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PHOTOSYNTHETIC ADAPTATION TO LIGHT INTENSITY IN LEAVES OF *ACER PSEUDOPLATANUS*

BY

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INTRODUCTION

During 1952 dry matter production in *Acer pseudoplatanus* L. (sycamore) growing under different light intensities was investigated. Two series of experiments were carried out, one under laboratory conditions at relatively low light intensities from daylight fluorescent tubes, and one under field conditions in the garden of the laboratory.

To investigate the capacity of sycamore to adapt itself to the intensity of the light field in which it is growing, determinations of the light intensity dependence of photosynthesis of the leaves, the relative chlorophyll content and the protein content of the leaves were made. Some of the findings which emerged will be discussed here.

METHODS

In each experimental series four light intensity classes were used. In the laboratory these different light intensities were obtained by varying the distance of the lamp sets from the plants, oustide by using gauze screens of different mesh width so that the sunlight was differentially intercepted.

In the indoor experiment the light intensities amounted to 24500-27000 ergs/cm^2 sec. (5000-5500 lux), 20000-22000 ergs/cm^2 sec. (4000-4500 lux), about 9800 ergs/cm^2 sec. (about 2000 lux), and 5880 ergs/cm^2 sec. (1200 lux), for 16 hours/day; in the outdoor experiment light intensities were roughly defined as 100 %, 75 %, 50 % and 25 % of the natural daylight. Owing to the irregular growth of the plants both indoors and outdoors it was impossible to ensure that every individual plant received the nominal amount of light energy, but in the indoor experiment the *average* amount of light energy in a light intensity class could be kept fairly constant. Thus, although the largest plants got somewhat too much light and the shorter ones too little, such differences were minimal. From the indoor experiment, leaves for the study of light-dependent properties were selected with the aid of a light meter. From the outdoor experiment the youngest fully expanded leaves from the various light intensity classes, were chosen.

TABLE 1

Rate of photosynthesis (expressed in relative units) at different light intensities in leaves from plants raised under various light intensities

Part A: Indoor experiment

Mean exposition light intensity in ergs/cm²/sec.	Nr. of leaf	Light intensity in ergs/cm²/sec. (watercooled high pressure mercurylamp)																
		2200	4400	6600	8800	11000	13200	15400	17600	19800	22000	24200	26400	28600	30800	33000	35200	37400
6100	1	1.3	2.5	3.4	3.8	3.9	3.9	3.9	3.9									
	2	1.2	2.1	2.8	3.0	3.0	3.0	3.0	3.0									
	3	1.3	2.2	2.8	2.9	2.9	2.9	2.9	2.9									
	4	1.3	2.4	3.0	3.3	3.5	3.6	3.7	3.7									
	5	1.2	2.3	2.7	2.7	2.7	2.7	2.7	2.7									
	6	1.0	1.9	2.5	2.6	2.6	2.6	2.6	2.6									
	7	1.2	2.1	2.7	3.1	3.1	3.1	3.1	3.1									
	8	1.4	2.2	2.7	2.8	2.8	2.8	2.8	2.8									
	9	1.1	1.7	1.8	1.8	1.8	1.8	1.8	1.8									
	10	0.8	1.3	1.5	1.5	1.5	1.5	1.5	1.5									
	av.	1.2	2.2	2.8	3.0	3.0	3.0	3.0	3.0									
15700	1	1.2	2.2	3.1	3.9	4.3	4.8	5.1	5.3	5.5	5.7	5.9	6.0					
	2	1.4	2.4	3.2	3.9	4.2	4.8	5.4	5.7	5.9	6.1	6.1	6.1					
	3	1.5	2.7	3.9	4.4	4.9	5.2	5.3	5.4	5.4	5.4	5.4	5.4					
	4	1.0	2.0	2.7	3.1	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3					
	av.	1.3	2.3	3.2	3.8	4.2	4.5	4.8	4.9	5.0	5.1	5.2	5.2					
27000	1	0.9	1.8	2.7	3.5	4.4	5.2	6.0	6.7	7.6	8.1	8.6	8.9	9.3	9.5	9.6	9.7	9.7
	2	0.9	1.7	2.2	2.7	3.2	3.7	4.2	4.5	4.7	4.8	4.9	5.0	5.0	5.0	5.0	5.0	5.0
	3	0.7	1.7	2.5	3.1	3.5	4.0	4.3	4.7	5.0	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
	4	0.7	1.2	1.6	1.9	2.2	2.4	2.7	3.0	3.1	3.3	3.4	3.4	3.4	3.4	3.4	3.4	3.4
	av.	0.8	1.6	2.2	2.8	3.3	3.8	4.3	4.7	5.1	5.4	5.6	5.7	5.8	5.8	5.9	5.9	5.9

av = average

TABLE 1, *continued.* Part B: Outdoor experiment

Exposition light intensity in % of daylight	Nr of leaf	Light intensity in ergs/cm² sec. (watercooled high pressure mercury lamp)															
		4400	8800	13200	17600	22000	26400	30800	35200	44000	48400	52800	57200	61600	66000		
25 %	1	1.3	2.1	2.6	2.6	2.6	2.6										
	2	1.6	2.8	3.3	3.6	3.7	3.7										
	3	1.6	2.8	3.6	4.0	4.2	4.2										
	4	1.5	2.6	3.4	3.8	3.9	3.9										
	5	1.4	2.8	4.0	4.8	5.1	5.2										
	av.	1.5	2.6	3.4	3.7	3.9	3.9										
50 %	1	1.4	2.4	3.4	4.3	5.0	5.7	6.1	6.3	6.3	6.3						
	2	1.4	2.6	4.0	4.9	5.3	5.6	5.7	5.8	5.9	5.9						
	3	1.9	3.6	4.7	5.8	6.7	7.3	7.6	7.8	7.8	7.8						
	4	1.4	2.6	3.5	4.2	4.6	4.8	4.9	4.9	4.9	4.9						
	av.	1.4	2.8	3.9	4.8	5.4	5.8	5.9	6.2	6.2	6.2						
75 %	1	1.4	2.5	3.2	3.9	4.4	4.8	5.3	5.6	5.9	6.1	6.4	6.6	6.6	6.6		
	2	1.4	2.5	3.2	3.8	4.5	5.0	5.3	5.5	5.6	5.6	5.6	5.6	5.6	5.6		
	3	1.4	2.5	3.4	4.1	4.7	5.2	5.6	5.9	6.1	6.2	6.4	6.5	6.5	6.5		
	4	1.2	2.6	3.5	4.2	4.8	5.4	5.9	6.3	6.6	6.9	7.1	7.2	7.3	7.3		
	5	1.4	2.4	3.1	3.6	4.2	4.5	4.7	4.9	5.1	5.3	5.4	5.4	5.4	5.4		
	6	1.4	2.6	3.8	5.0	5.9	6.7	7.5	8.2	8.7	9.0	9.4	9.7	9.7	9.7		
	7	1.2	2.5	3.7	4.7	5.7	6.7	7.6	8.4	9.2	9.7	10.4	11.0	11.5	11.5		
	av.	1.3	2.5	3.4	4.2	4.9	5.5	6.0	6.4	6.6	7.0	7.2	7.4	7.5	7.5		

av = average

Measurements of the light intensity dependence of photosynthesis in detached leaves were made using a Kipp diaferometer. For this purpose, a single leaf at a time was enclosed in an assimilation chamber and illuminated from one side with a watercooled high pressure mercury-lamp. With the aid of a water bath the temperature of the assimilation chamber was held at the constant level of 20° C. Water vapour saturated air with a CO_2-content of 5 % passed through this chamber. For the first determination a relatively high illumination-intensity was chosen in order to secure light saturation, a period of about $^3/_4$ of an hour being required to reach the maximum assimilation level (induction phenomena). By gradually lowering the light intensity, it was then possible to determine quickly the remaining points on the intensity curve. Respiration was measured as CO_2-exchange in the dark.

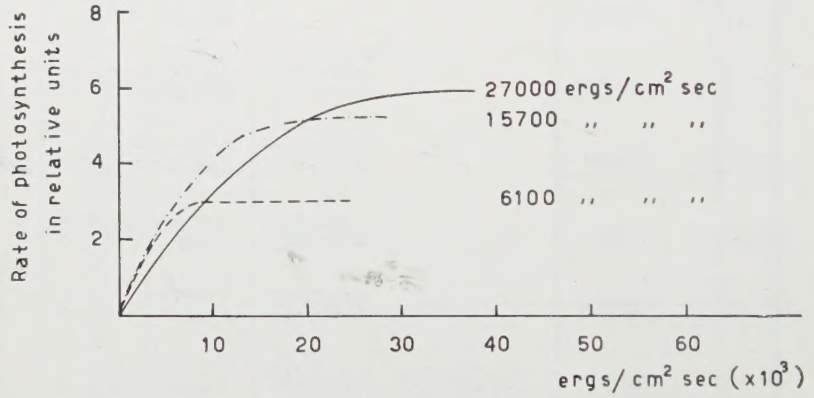

Fig. 1. Indoor experiment

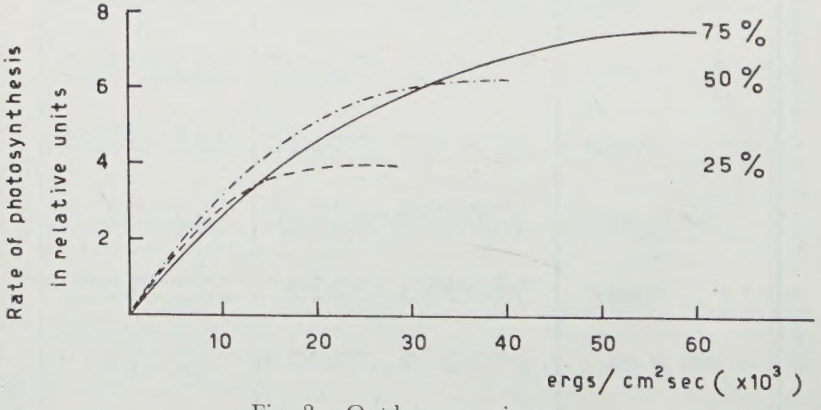

Fig. 2. Outdoor experiment

Figs. 1 and 2. The relations between photosynthesis and light intensity. These curves are drawn from averages of data in Table 1. With increasing exposition light intensity the saturation intensity and maximum rate of photosynthesis increase.

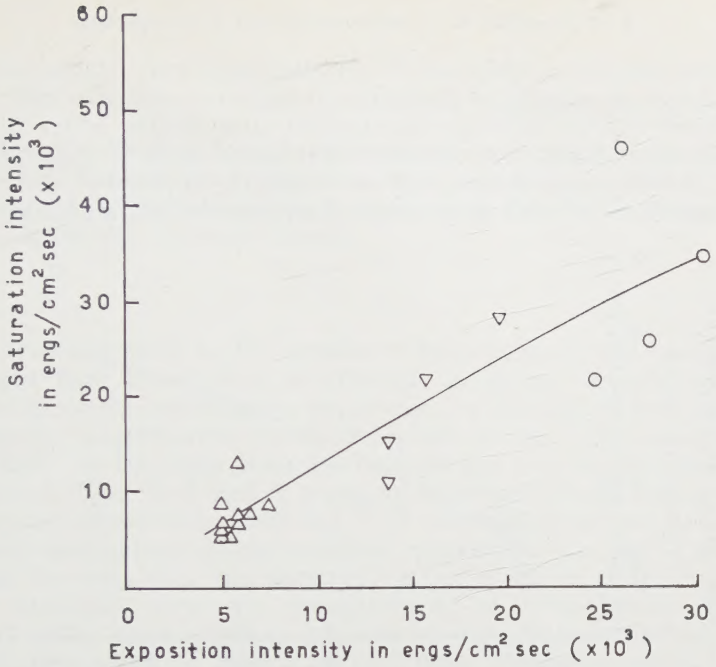

Fig. 3. Indoor experiment

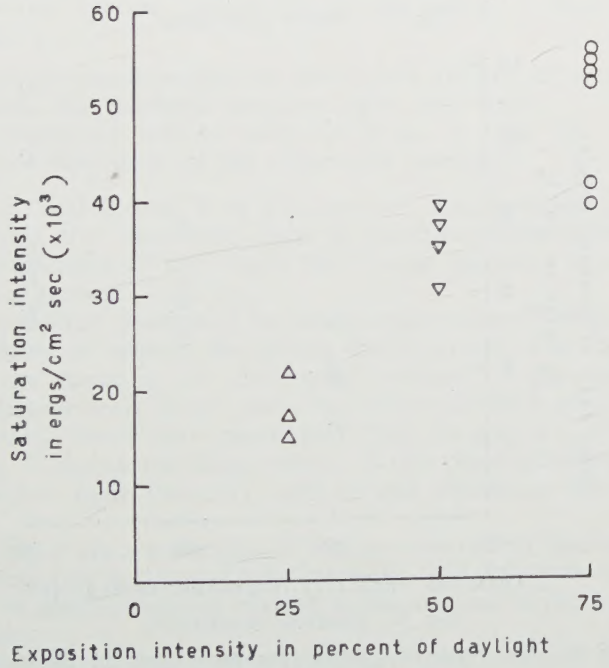

Fig. 4. Outdoor experiment

Figs. 3 and 4. The relation between saturation light intensity and exposition light intensity.

Indoor experiment:
△ exposition intensity 6100 ergs/cm²/sec.
▽ exposition intensity 15700 ergs/cm²/sec.
○ exposition intensity 27000 ergs/cm²/sec.

Outdoor experiment:
△ exposition intensity 25 % of daylight.
▽ exposition intensity 50 % of daylight.
○ exposition intensity 75 % of daylight.

Determinations of chlorophyll concentration were made using the same leaves. A number of discs (3 to 9) were cut out of a leaf with a punch and quickly killed by dipping them into boiling water for some seconds. They were then extracted repeatedly with 60 % ethanol at 70° C to remove the chlorophyll as completely as possible, the extract made up to 25 ml with 60 % ethanol and the extinction value of this

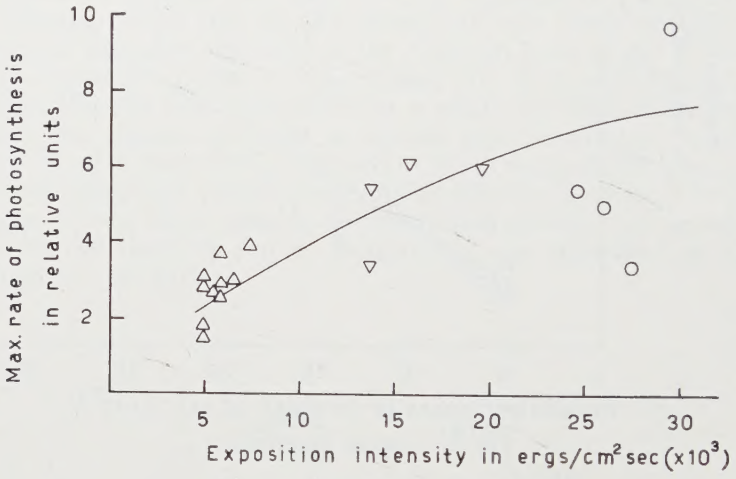

Fig. 5. Indoor experiment

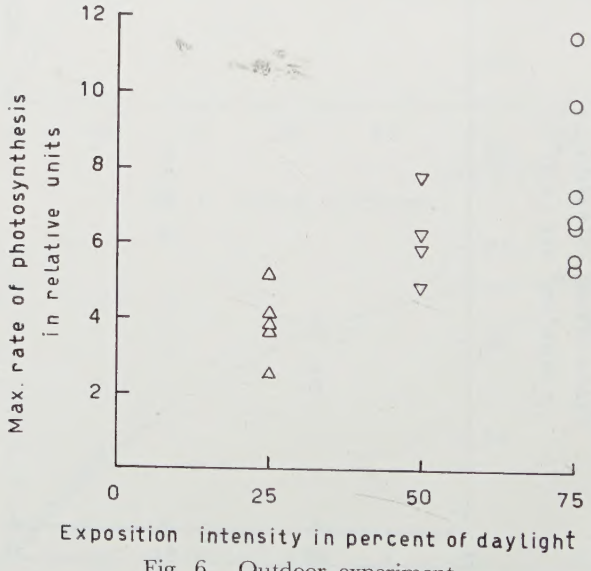

Fig. 6. Outdoor experiment

Figs. 5 and 6. The relation between maximum rate of photosynthesis and exposition light intensity.

The spreading of the different points is partly due to the different chlorophyll concentrations (cf. fig. 7). For explanation of symbols, see figs. 3 and 4.

extract determined with a Bleeker extinction meter at a wavelength of 665 mμ. The resultant value was taken as a measure of the chlorophyll content of the leaves. Finally, micro-protein determinations were made, again on the same leaves.

Results

Sycamore appears to be capable of adaptation to the intensity of the light field in which it is growing, in several respects. Certain properties of the assimilation apparatus, its chlorophyll content and its protein content were found to depend on this light intensity.

In figs. 1-6 the light intensity dependence of CO_2-assimilation is illustrated. Figs. 1, 3 and 5 represent leaves of plants belonging to the indoor experiment, while figs. 2, 4 and 6 illustrate the behaviour of leaves from plants of the outdoor experiment (cf. also Table 1). It can be seen that the intensity curves of leaves adapted to low light intensities show the characteristics of "Blackman curves" (fig. 1) while those of leaves adapted to high light intensities show the characteristics of "Bose curves", (fig. 2) with a gradual transition from one type to the other (cf. Rabinowitch, 1951).

At least two specific values can be derived from these intensity curves, viz.:

1° The saturation intensity, i.e. the light intensity, at which photosynthesis has reached complete light saturation.
2° The maximum rate of photosynthesis, i.e. the rate of photosynthesis obtaining at the saturation intensity.

From figs. 1 and 2, and 3 to 6 it appears that both the saturation intensity and the maximum rate of photosynthesis increase with increasing intensity of the "light field"—the intensity at which the leaves have grown.

The chlorophyll content of the leaves also varies in relation to the light intensity at which the plants have grown. The higher this intensity, the lower is the chlorophyll content of the leaves. This could readily be seen by eye from the colour of the leaves; "shadow leaves" were a fresh dark green and "sun leaves" a pale-yellowish green. Fig. 7 shows the dependence of the chlorophyll content on the exposition light intensity, and clearly illustrates this adaptive effect.

Finally, fig. 8 gives some idea of the increase in leaf thickness under the influence of an increasing light intensity, and the rise in dry weight/cm^2 must be ascribed almost entirely to an increase in the non-protein compounds.

The points in this graph were computed from a smoothed curve of mg protein per g dry weight of the leaf plotted against the exposition light intensity and a curve of mg dry weight of the leaf per cm^2 against the exposition light intensity. While the percentage of protein in the dry weight of the leaf decreases with increasing exposition light intensity, the dry weight per cm^2 of the leaf increases correspondingly. It

follows that the absolute amount of protein per cm^2 in the leaf is hardly influenced by the intensity of the light field.

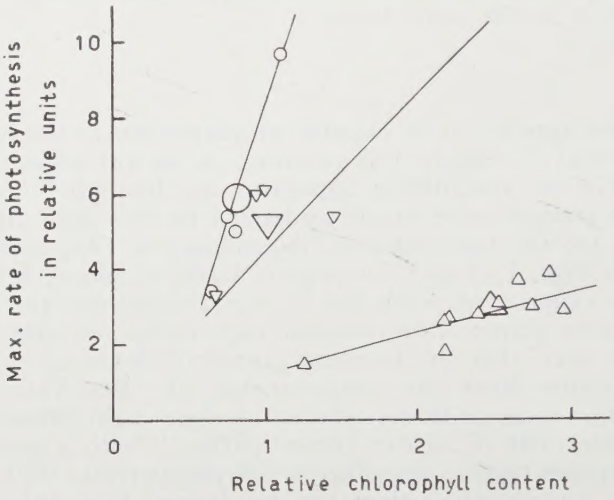

Fig. 7. Indoor experiment. The relation between maximum rate of photosynthesis and relative chlorophyll content.
For explanation of symbols, see figs. 3 and 4.

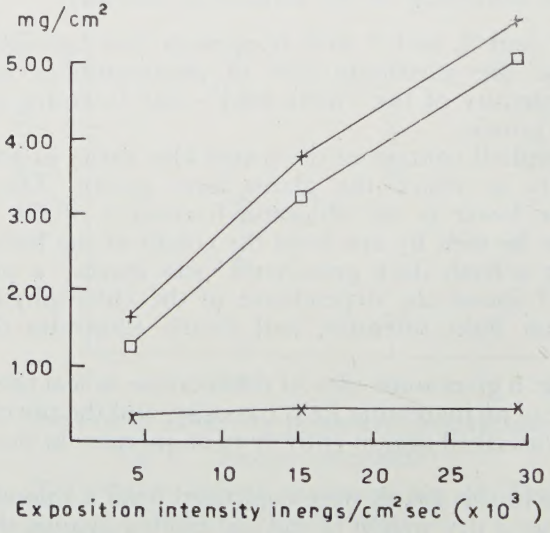

Fig. 8. Indoor experiment. The relation between leaf dry weight and exposition light intensity

× — Amount of protein in mg/cm^2.
□ — Amount of non-protein-compounds in mg/cm^2.
\+ — Total dry weight of the leaf in mg/cm^2.

Discussion

It appears from literature that adaptation phenomena of plants in relation to light are well known. Those of the chlorophyll system in particular have been frequently investigated. Before 1918 it was generally held that during photosynthesis there is a continuous destruction and resynthesis of chlorophyll. In 1918, however, Willstätter and Stoll claimed that in adult leaves chlorophyll was present in a fixed form and in a definite amount. On the authority of these investigators this was generally accepted for a long time, although results of later experiments have repeatedly raised doubts about the generally accepted stability of chlorophyll. For instance, Bukatsch (1940), Wendel (1940) and Henrici (1919) found regular variations in the chlorophyll content of leaves during the day. Since, however, they relate chlorophyll content to the fresh weight of the leaves, the data are of limited relevence. Montfort (1941) distinguishes two types of plants: the photostable type, reacting upon increasing intensity of the light field by increasing its chlorophyll content and *vice versa*, and the photolabile type, reacting upon increasing intensity of the light field by decreasing its chlorophyll content. According to these views sycamore as demonstrated by the present experiments should belong to the photolabile type. Montfort ascribes the diminution in chlorophyll content with increasing light intensity to its destruction by ultra-violet radiation. However, the mechanism of the production of chlorophyll under these circumstances is not clear, and Montfort does not discuss the question of a physiological equilibrium.

The existence of a dynamic equilibrium has, however, been accepted by various investigators including Stålfelt (1927), Zacherowa (1929), Schertz (1929), Sjöberg (1931), Roux and Husson (1952). Schenk (1952-1953) found a yearly periodicity in the amount of chlorophyll in the cortex of *Tilia*, *Fagus* and *Populus*, which fact he ascribes to an endogenous rhythm in the plant. An interesting contribution to this subject was made by Randall (1953) in his study on water relations and chlorophyll content of forest herbs in Southern Wisconsin. He found that the different herbs of the forest border show a gradual drop in chlorophyll content of their leaves from high forest to savanna, i.e. from low to high light intensity conditions.

It seems, therefore, that, although some minor indications point to the existence of a reversible equilibrium between chlorophyll content and light intensity during growth, the existence of such an equilibrium is far from being sure and further investigations on this subject are now in progress.

Equally little appears to be known about other adaptation phenomena in the photosynthetic apparatus. There are many references in the literature to sun leaves and shade leaves, sun leaves having the capacity of using more of the sunlight for photosynthesis than shade leaves, and this view is fully in agreement with our findings. As to the reason for variation in photosynthetic capacity, however, it is not possible at this stage to be definite, although Rabinowitch (1945)

ascribes such differences to an enzymatic factor. The present experiments demonstrate that the difference in capacity cannot at any rate be correlated with the thickness of the leaf, since it has been established that the increment in thickness is almost entirely accomplished by the formation of non-protein compounds.

The relation between chlorophyll content and maximum rate of photosynthesis is complex. Thus, while the correlation between the maximum rate of photosynthesis and the chlorophyll content of the different light classes is negative, within each light class this correlation seems to be positive. It is possible that hereditary factors are involved here and this suggestion is also being further investigated.

SUMMARY

Some preliminary experiments on light adaptation phenomena in *Acer pseudoplatanus* L. (sycamore) are described. First year seedlings were grown under four different light intensities. The light intensity dependence of photosynthesis, and the relation between exposition light intensity and both chlorophyll content and protein content were measured on single leaves.

1. It appeared from the photosynthesis curves that, with increasing exposition light intensity, the saturation light intensity as well as the maximum rate of photosynthesis, increases.
2. The chlorophyll content/cm^2 decreases with increasing exposition light intensity.
3. Within each light intensity class, the maximum rate of photosynthesis seems to be directly correlated with the chlorophyll content.
4. In strong light, the leaves are thicker, however, without conspicuous increase in protein content.

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PEROXYDASE CONTENT OF DWARF TYPES AND GIANT TYPES OF PLANTS

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1. Introduction

Several publications report an abnormal content of the enzyme peroxydase in dwarf types and giant types of plants. In the former case the content is said to be greatly increased, in the latter greatly decreased. In most cases however, the quantity of the enzyme was only approximately estimated.

As the Genetic Laboratory of the university at Groningen had *Phaseolus* dwarf plants available it seemed desirable to determine the enzyme content in a more quantitative way. The researches took place at the Botanical Laboratories at Groningen and Leiden.

In 1935 VAN OVERBEEK published a study on the dwarflike growth of maize seedlings. The dwarfs differed from the normals in that the mescotyl remained short. The coleoptile was in both cases of the same length. His material was exceedingly suitable for a comparative physiological study because he worked with samples of seedlings which produced 50 % normals and 50 % dwarfs. This was due to the fact that the dwarf property was based on one genetically recessive factor. The research led van Overbeek to conclude that an equal quantity of growth substance was formed in either race, but that in the case of the dwarfs more destruction took place during the transport in basal direction. The cell elongation in the mesocotyl was thus differently influenced. Besides, van Overbeek found a higher catalase and peroxydase content in dwarfs than in normals. The author suggested that these enzymes were responsible for the inactivation of the growth substance, the latter being oxidized.

The reverse, i.e. giant growth, has been described and examined closer by DE HAAN and GORTER (1936). From *Pisum* crosses a so-called slender pea could be grown which was more than three metres tall. The genetic condition for this was that a certain two recessive factors should be homozygously present. So here too the phenomenon was genetically determined. Researches as to the catalase activity showed that it was subnormal in the slender pea. Moreover they thought it probable that less growth substance was inactivated in the slender pea than in normal types. Like van Overbeek they did not prove that the inactivation was caused by the oxidizing enzymes.

Dwarf types of *Epilobium hirsutum* L. have been described and

examined e.g. by Michaëlis (1943). Beside dwarf types there were examples of growth stimulation to be found in the *Epilobium* crosses which may be ranged under the genetic phenomenon of heterosis. So the symptoms were also genetically controlled, though here it was a question of so-called plasmatic heredity. The physiological behaviour of these plants has been investigated by Ross (1941, 1942, 1948). His work clearly appears to have been inspired by van Overbeek's train of thought. In his investigations correlation was found between the inhibition or stimulation of growth and the peroxydase content. In plants with inhibited growth the peroxydase content was higher, in plants with stimulated growth it was lower. From the research it has not become clear whether there was a causal relation between peroxydase content and rate of growth.

The lack of a bacterium symbiont in *Ardisia crispa* (Thunb.) A.DC. results in a cripple of the same plant. This has been described in detail by de Jongh in 1938. He showed that, very probably, the catalase content is higher in the cripple than in the normal plant and that peroxydase is only demonstrable in the dwarf.

2. Method of determination of peroxydase

To determine the peroxydase content the method of Derx (1942 *a* and *b*) has been used. His method is based on the oxydation of the colourless o-tolidine, which has a blue reaction product. Adding an extract containing the enzyme peroxydase to a mixture of solutions of o-tolidine and hydrogen peroxide the blue oxidation product of o-tolidine will appear. If to this solution ascorbic acid is added the blue reaction product will be reduced to the colourless substance, which will remain colourless until the whole of the ascorbic acid will have been oxidized. Then the solution will suddenly turn blue. The time necessary for the appearance of the blue colour is, with a certain quantity of ascorbic acid, inversely proportional to the activity of the peroxydase enzyme. So the method is chronometrical.

Standardizing the conditions Derx has used this reaction for a quantitative peroxydase test. To start the reaction we always added a temperature-conditioned mixture of 1 ml H_2O_2 (0.1 n), 0.5 ml o-tolidine (0.05 %) and 22.5 ml citrate-buffer solution to a mixture of 0.5 ml ascorbic acid (0.01 n), 1 ml enzyme solution and 4.5 ml buffer solution. According to the activity of the enzyme solution the quantity of the ascorbic acid solution and the enzyme solution may be varied, provided that the total volume remains 30 ml. From the time the blue colour appears one may calculate the quantity of the enzyme solution necessary for reducing one millimol H_2O_2 in one minute. This unit Derx calls "Unité Normale de Peroxydase" (U.N.P.). As in the preparation of the enzyme solution a definite quantity of the leaves is used, it is possible to calculate the enzyme activity on the fresh weight of the plant material.

The standardizing conditions for the Derx reaction were: 1. limitation to no more than 5 % conversion of the added H_2O_2. 2. ph 5.

3. temperature 25° C. 4. certain definite concentrations of H_2O_2 and o-tolidine.

Derx's method has considerable advantages over other methods of peroxydase determination described. An important fact is that his method of enzyme preparation includes a purification as regards to the reducing substances in the plant extract. The enzyme is precipitated by aethyl alcohol (96 %) and the residue is dissolved in a buffer solution.

Derx took 10 to 20 gram of plant material for his enzyme preparation. For our own use we often took no more than half a gram of plant material. The quantitative preparation of the residue thus demands more care, but it can very well be done with the use of small filter cups (Schott 63 G 3) 2 centimetres in diameter. There is no reason why in this way the determination should be less accurate.

In all cases the enzyme content was calculated per gram fresh weight of the plant material. Young leaves were always used for the enzyme preparations.

3. Results

Tropaeolum majus L.

Of this species there exist varieties which remain low near the ground and which do not climb. Comparing them with the normal climbing type their habitus strikes us as being stunted in growth and their leaves remain noticeably smaller. The small type being traded under the name of "nana compactum", "Tom Pouce", etc. Though the differences between these varieties are small the plants were used to test the methodology of the peroxydase determination. The results were the following.

variety	U.N.P./gram fresh weight
climbing type	0.015 ± 0.0006 $(n = 12)$
stunted type	0.023 ± 0.002 $(n = 6)$

Thus the peroxydase content of the stunted type was greater.

Phaseolus crosses

Geerts (1949), in his thesis, describes *Phaseolus* crosses in which in the F_1-generation giant types occurred besides dwarfs. The dwarf types were exceedingly small. Their habitus was badly stunted, their leaves were small and in many cases the growth of leaf-vein and -tissue was irregular. In the giant type the leaf was extraordinarily large. The phenomenon is not yet completely genetically explained, but possibly a plasmatic heredity plays a role here.

Table 1 gives the peroxydase contents found by the present author in the leaves of various types that occur in these cross-breedings. First of all the enzyme content of the two parents is given. Next that of types that have grown bigger than the parents and lastly of dwarf

types in the F_1- and F_2-generation. The F_2-generation also contained semi-dwarfs.

Table 1 clearly shows the extraordinarily high peroxydase content in the leaves of the dwarf types. The enzyme content of the semi dwarfs lies, in spite of their climbing type, in between normals and dwarfs, whereas in giant types (except for number 3192) the content

TABLE 1

Peroxydase content of *Phaseolus* plants; explanation: see text

		mean value U.N.P./gram $\times 10^{-3}$	stem length cm	number extractions
Phaseolus vulgaris P_1	302	70	40	5
Phaseolus multiflorus P_2	308	113	50	3
F_1 giant type	3191	36	65	9
F_1 giant type	3192	128	65	12
F_1 giant type	316	33	60	1
F_1 dwarf	3195	2400	25	3
F_1 dwarf	3199	3200	25	3
F_2 dwarf	32867	630	30	1
F_2 semi-dwarf	32607	130	200*	2
F_2 semi-dwarf	32926	260	170*	3

* Semi-dwarfs stand quite apart as to type. They are thinly built, climbing plants, but their leafs show the habitus of the dwarf type.

is below the normal. It looks as if the peroxydase content might be taken as a measure for the degree of dwarfing of the plant.

With such enormous differences occuring in the enzyme activity one wonders whether it is always the same enzyme one is dealing with. Therefore it was investigated whether in extracts of peroxydase from cripples the optimum of the Derx reaction occurred with the same hydrogen peroxide concentration and the same acidity as in extracts of peroxydase from normal plants. With the o-tolidine concentration of 8.3×10^{-3} per cent in the reaction mixture, in both cases the optimum hydrogen peroxide concentration was about 2.5×10^{-3} mol. (Compare with Derx's data). To determine the optimum acidity of the enzyme reaction a series of buffer solutions according to Mc Ilvaine was used. In both cases the optimum lay at pH 5.2 and 5.3. So it seems likely that in these cases the enzymes are the same.

Crosses of Nicotiana Tabacum L.

In 1944 I received seeds of tobacco crosses from the late professor J. A. Honing from Wageningen. For the genetical background of this material the reader is referred to Honing (1939). Some peroxydase extracts have been made of leaves from two types of the *Kloempang* dwarf race, one type being greater than the other in height as well as in size of the leaves. These two types of *Kloempang* dwarf race grow by heterozygosity from one and the same sowing. Apart from these, some determinations were carried out in extracts from the leaves of a necrotic tobacco dwarf, the plants of which remained exceedingly small. Table 2 gives a survey of the results.

TABLE 2

Peroxydase content in several tobacco plants; explanation: see text

	mean value U.N.P./gram $\times 10^{-3}$	stem length cm	number extractions
Kloempang dwarf race 1561–120			
tall type	9	120	2
small type	12	40	2
necrotic dwarf	100	5	8

Though these observations were few, a connection between the peroxydase content and the degree of dwarfing is evident.

Zea Mays L.

In 1944 a number of dwarf maize cobs happened to be available in the Botanical Laboratory at Leiden, received by prof. L. G. M. Baas Becking in 1938 from J. van Overbeek. Due to the age of the material the maize grains had little germinating capacity. One cob was an exception to the rule however, and after sowing produced sound maize seedlings, 50 % normals and 50 % dwarfs. Table 3 gives the results of peroxydase determinations of the mesocotyl, the coleoptile and the primary leaf of these plants.

TABLE 3

Peroxydase content of the mesocotyl, coleoptile, and primary leaf of maize seedlings.

	U.N.P./gram fresh weight $\times 10^{-3}$ normals	U.N.P./gram fresh weight $\times 10^{-3}$ dwarfs	ratio U.N.P. dwarf/normal
mesocotyl	32	49	1.5/1
coleoptile	31	53	1.7/1
pr. leaf	27	28	1.0/1

For one peroxydase extract 12 to 14 coleoptile or mesocotyl pieces or primary leaves have been used. Though determinations could be made only with one plant series—which makes the conclusions somewhat doubtful—the result confirms van Overbeek's informations. Van Overbeek (1935) found a proportion of the catalase content of the dwarf to that of the normal of 1.5 and 1.9 to 1. The proportion between the peroxydase contents in Table 3 is in accordance with it.

If we calculate the enzyme content on the basis of dry weight, the differences between dwarfs and normals become even greater. The differences remain, even when the enzyme content is calculated on the basis of the water content of the tissues.

4. Discussion

As far as the small numbers of experiments allow a conclusion it has been shown that the peroxydase content of the leaves is in all cases correlated with the degree of dwarfing of the plant. The same

was the case with the tendency to form giant types. Determinations with coleoptile and mesocotyl pieces of dwarf maize show the same picture. In dwarfs of *Phaseolus* cross-breedings the peroxydase content was extremely high. It would seem useful to compare these figures with the content of this enzyme in other plants.

Derx (1942*a*) as well as Willstätter (1928) give an account of the enzyme content in various plants. Among them *Cochlearia armoracia* L. has the highest content (U.N.P./gr 0.68 fresh and 2.63 dry). Though the methods of determination of the two authors differ, a comparision is very well possible because both state the enzyme content of *Cochlearia*. In both cases it can be expressed in millimol H_2O_2 converted per minute and per gram dry weight. Derx: 2.6 millimol H_2O_2/minute/gr. dry weight. Willstätter: 2.2 millimol H_2O_2/minute/gr. dry weight. These two figures are well in accordance with each other, considering that Derx worked with a reaction temperature of 25° C. and Willstätter at 20° C.

So, in comparison with the enzyme content in other plants the peroxydase content in *Phaseolus* dwarfs is shown to be extraordinarily high.

An open question is what significance should be attached to a higher or lower peroxydase content. According to Ross a high content is a symptom of an all-round increase of dissimilative metabolism of the plant. It should be examined whether and to what extent an increase or decrease of the enzyme content affects other enzymes than the peroxydase. Furthermore the question remains whether the peroxydase content is due to or is the cause of the dwarf growth. It is very well possible that the effect is a secundary one.

As remarked before, determinations of the pH-optimum and the optimum of the hydrogen peroxide concentration showed that in bean dwarfs very likely the same agent was isolated as in normal bean plants.

Because in some cases, as mentioned above, the giant growth may be considered as a heterosis phenomenon, there is a possibility that by means of the peroxydase test data will become available concerning the physiological aspect of the problem of heterosis.

SUMMARY

The information given in the literature concerning the peroxydase content of dwarf plants and giant plants has been quantitatively tested in various types of plants. The results were consistent with the conception that dwarf plants have a peroxydase content which is higher than normal and that giant types give exactly the reverse picture. In *Phaseolus* dwarfs an exceedingly high peroxydase content was found.

ACKNOWLEDGMENTS

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HEMMSTOFFE UND WACHSTUM

VON

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Zahlreich sind die in den letzten Jahren durchgeführten Untersuchungen über den Einfluss pflanzlicher Hemmstoffe auf die Keimung der Samen. Uebereinstimmend stellen sie alle fest, dass diese aus Samen, Früchten oder Sporen gewonnenen Hemmstoffe die Keimung der Samen resp. Sporen verzögern, ja unter Umständen unterbinden, und dass sie das Wachstum der Keimpflanzen retardieren (EVENARI, 1949; FROESCHEL, 1940; NIEMANN, 1952).

Unbeantwortet indess ist bis heute die Frage, wie Pflanzen wachsen und sich entwickeln, wenn sie weit über das Keimlingsstadium hinaus, eventuell bis zur Blütezeit, unter dem kontinuierlichen Einfluss von Hemmstoffen gehalten werden.

Die folgenden Zeilen berichten über derartige Versuche, die hauptsächlich an *Hordeum*, *Pisum* und *Agrostemma* durchgeführt wurden.

Die verwendeten Hemmstoffe stammten wie in früheren Untersuchungen aus den Saaten von *Beta saccharifera*, aus denen sie durch 24-stündige Extraktion eines Teiles Saat durch 4 Teile Wasser gewonnen wurden. Um einen Hemmungsfaktor osmotischer Natur auszuschalten, wurden diese Extrakte demineralisiert. Aus dem zugehörigen Trockenpräparat wurden dann durch Auflösen in Knopscher Nährstofflösung die einzelnen Hemmstoffkonzentrationen bereitet.

VORVERSUCHE MIT *Triticum*

Für diese Versuche, die auf das Jahr 1945 zurückgehen, wurde als Hemmstoffquelle das Konditionswasser einer Mühle verwendet. Dieses Wasser, das aus maltechnischen Gründen mit dem Getreide etwa eine Minute in Berührung gebracht und dann von den Mühlen in grossen Mengen verworfen wird, stellt eine bequem erreichbare und reichlich fliessende Hemmstoffquelle dar, genau so wie das Weichwasser der Mälzereien. 10 bis 20-fach konzentriertes Konditionswasser gibt ausgezeichnete Hemmeffekte und wenn es auch höchst wahrscheinlich ist, dass zufolge reichlichen Gehaltes an NH_4Cl ein osmotischer Faktor in den Hemmungseffekt eingeht, so ist—in Analogie mit der hemmenden Wirkung demineralisierten Weichwassers—als sicher anzunehmen, dass im Konditionswasser ebenfalls eine spezifische organische Substanz ihre hemmende Wirkung ausübt (FROESCHEL, 1955).

Kultiviert man nun Weizen auf einem 10-fachen Konzentrat dieses Weizenwaschwassers, so zeigt sich nach einem Monat ein erstaunlicher Unterschied zu jenem Weizen, der auf Wasser gezogen wurde.

Von den in Abb. 1 dargestellten Pflanzen wurde a kultiviert auf Wasser, b und c auf einem 10-fachen Konzentrat des Konditionswassers. Wiewohl a und b im gleichen Entwicklungsstadium sind (zwei Blätter), erreicht b nur ein Viertel der Höhe von a. Bei c ist die Hemmung noch viel weiter gegangen: die Pflanze ist nicht nur in der Grösse sondern auch in der Entwicklung zurück, da sie nur die Spitze des ersten Blattes zeigt, das eben die Koleoptile durchbrochen hat. Sie ist ein Zwerg geblieben.

Der Wunsch, weiteres Material zu dieser Frage zusammenzutragen, führte zur Wiederaufnahme des Themas, wobei jedoch nur mehr demineralisierte Beta-Hemmstoffe zur Anwendung kamen.

Versuche mit *Hordeum*

Die Gerste wurde in Glasröhrchen (Höhe 6 cm, Durchmesser 2,5 cm) kultiviert, die mit Seesand gefüllt waren. Die Befeuchtung des Kulturmediums geschah bei den Kontrollen durch Knopsche Nährlösung, bei den Versuchen durch verschieden konzentrierte Hemmstofflösungen. 10 Versuchspflanzen per Konzentration.

Die Ergebnisse entsprechen durchaus den oben geschilderten Vorversuchen mit Weizen. Nach einer Kultur von 5 Wochen ergibt sich ein Resultat, das durch die Abb. 2 besser dargestellt wird als durch ein langatmiges Protokoll.

Durch Anwendung stärkerer Konzentrationen konnte die Hemmung noch weiter getrieben werden, da zwischen den verwendeten Konzentrationen und jener, die die Samenkeimung überhaupt verhindert, Interpolationen möglich sind. Doch wollen wir vorsichtiger Weise diese noch weiter gehenden Hemmungen nicht ausschliesslich den Hemmstoffen zuschreiben, da bei diesen stärkeren Konzentrationen die Alkalinität des Präparates (pH 8) schon ins Spiel treten kann.

Versuche mit *Pisum*

Pisum wurde in der selben Weise kultiviert wie *Hordeum* und erwies sich gegenüber den gleichen Hemmstoffen als viel empfindlicher. Die Konzentrationen 2,5 % und 1,25 % wurde je drei mal mit je 10 Pflanzen getestet.

Abb. 3 zeigt das Ergebnis eines dieser Versuche nach 20 Tagen. Die Konzentration von 1,25 % reduziert das Längenwachstum bereits um mehr als die Hälfte gegenüber den Kontrollen, während eine Konzentration von 2,5 % die meisten Samen ungekeimt lässt und nur dem einen oder andern ein Zwergwachstum gestattet.

Abb. 4 zeigt detailliert, dass die Hemmstoffe durch Verminderung sowohl der Länge als der Zahl der Internodien nicht nur einen retardierenden sondern auch einen formativen Einfluss ausüben. Er tritt bei den Zwergpflanzen ganz besonders hervor.

Versuche mit *Agrostemma*

Um mit der begrenzten Menge demineralisierter Hemmstoffe zurecht zu kommen, wurden die Agrostemma-Samen zu je 16 in flachen Tonschalen angebaut. In Anwendung kamen die Konzentrationen 5 %, 2,5 %, 1,25 %, 0,62 % und 0,31 %. Eine Hemmstoffkonzentration von 5 % lässt eine Samenkeimung überhaupt nicht zu [1]). Bei der Konzentration 2,5 % entwickeln sich Pflanzen, die noch nicht die Hälfte der Höhe der Wasserkontrollen erreichen.

Abb. 5 zeigt, dass sowohl die Länge der Internodien als auch die Dicke des Sprosses, die Breite der Blätter, die Entwicklung des Wurzelsystems einer starken Hemmung unterliegen. Auch die Gesamtentwicklung ist zurück: während die Normalpflanzen nach dem 9. Blattpaar (Kotyledonen mitgerechnet) die Blüte entwickelt haben, ist bei der Hemmstoffpflanze das achte Blattpaar noch nicht entfaltet.

Leider konnte das Blütenstadium der Hemmstoffpflanze nicht abgewartet werden, da sie gleich einem zweiten Exemplar abzusterben drohte.

Das kommt von der angewendeten Technik her, die, ohne im Uebrigen die Richtigkeit unserer Resultate in Frage zu stellen, doch unvollkommen ist. Bei dauerndem Zusetzen von Hemmstofflösungen zum Boden ist nämlich die Pflanze nicht einer konstanten sondern einer stetig steigenden Hemmstoffkonzentration ausgesetzt, die schliesslich zum Tode führen kann.

Neue Versuche sollen daher mit einer Technik unternommen werden, die eine konstante Hemmstoffkonzentration gewährleistet. Von solchen Versuchen darf man sich mit einiger Wahrscheinlichkeit das Entstehen blühender Zwergexemplare erwarten.

Was die Konzentrationen 1,25 %, 0,62 % und 0,31 % anlangt, so ergaben sie teils mehr oder minder gehemmte Exemplare, teils aber solche, die den Wasserkontrollen sogar voraus waren. Aus letzterem Faktum kann man eine Bestätigung der öfters geäusserten Ansicht herauslesen, wonach Wuchs- und Hemmstoffe leicht sollen ineinander übergehen können.

Diskussion

Unsere vorstehend beschriebenen Versuche lehren, dass Pflanzen, die von der Samenkeimung ab durch Wochen oder Monate unter dem Einfluss von Hemmstoffen gehalten werden, eine dreifache Reaktion zeigen:

1) Eine Verlangsamung des Wachstums.
2) Eine Verkleinerung von Stamm, Blatt und Wurzel-Verkleinerung die sehr fein abgestuft werden kann und die bis zur Bildung von Zwergformen gehen kann.
3) Ein Zurückbleiben in der Entwicklung, indem in der gleichen Zeit nicht nur kleinere sondern auch weniger Organe gebildet werden.

[1]) Die Samen konnten aber nach einmonatlicher Hemmung auf mit Wasser befeuchtetem Filterpapier zur Keimung gebracht werden.

Fig. 1

Abb. 1. Weizenpflanzen. *a.* kultiviert auf Wasser, b. und *c.* auf 10-fach Konzentrierten Mühlenwaschwasser dieses Weizens. — Versuchsdauer 30 Tage. — 1/3 der nat. Grösse

Abb. 2. *a.* Gerste kultiviert auf Knopscher Nährlösung. *b.* Gerste kultiviert auf einer 2,5 %-igen Hemmstofflösung. Koleoptile, Blätter und Wurzelsystem erscheinen gehemmt

Abb. 3. Versuch mit *Pisum*, photographiert nach 20 Tagen, 1/6 der nat. Grösse Obere Reihe: auf Wasser. Mittlere Reihe: Hemmstofflösung 1,25 %. Untere Reihe: Hemmstofflösung 2,5 %

Abb. 4. Je eine Pflanze der drei Reihen der Abb. 3. Nat. Grösse

Abb. 5. Zwei Exemplare von *Agrostemma Githago*, 80 Tage nach Aussaat. *a.* kultiviert auf Erde mit Wasser, *b.* auf Erde mit 2,5 %-iger Hemmstofflösung. 1/3 der nat. Grösse. (Bei *a.* ein Blattpaar abgefallen)

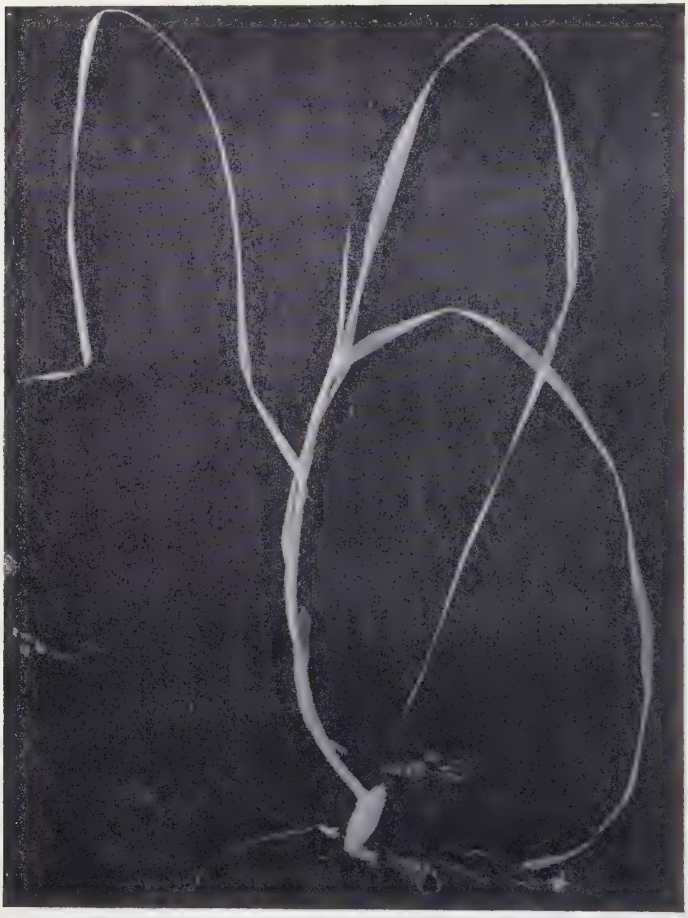

Fig. 2a

Fig. 2b

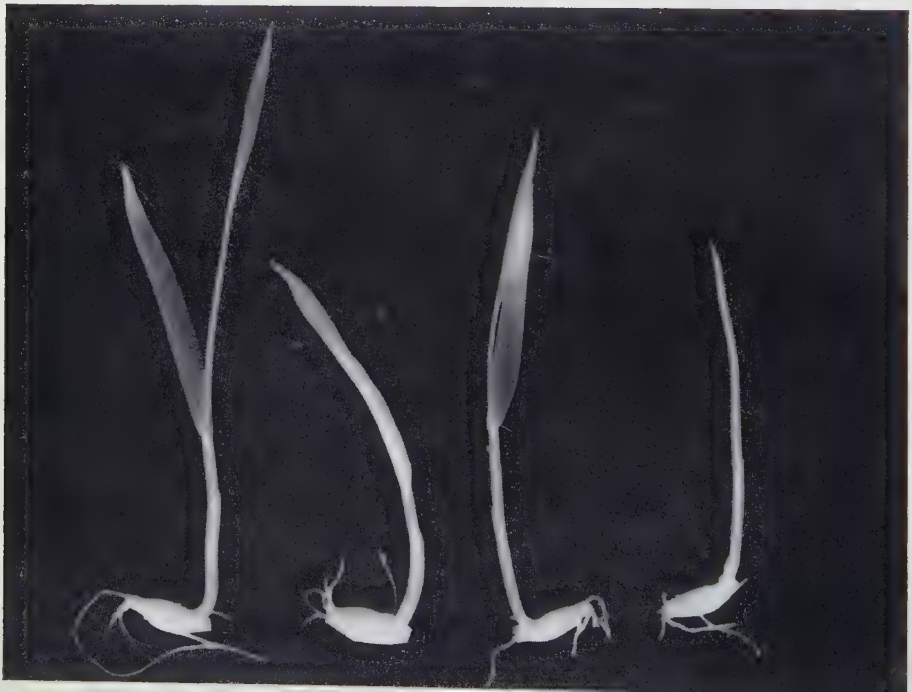

Fig. 3

Fig. 4

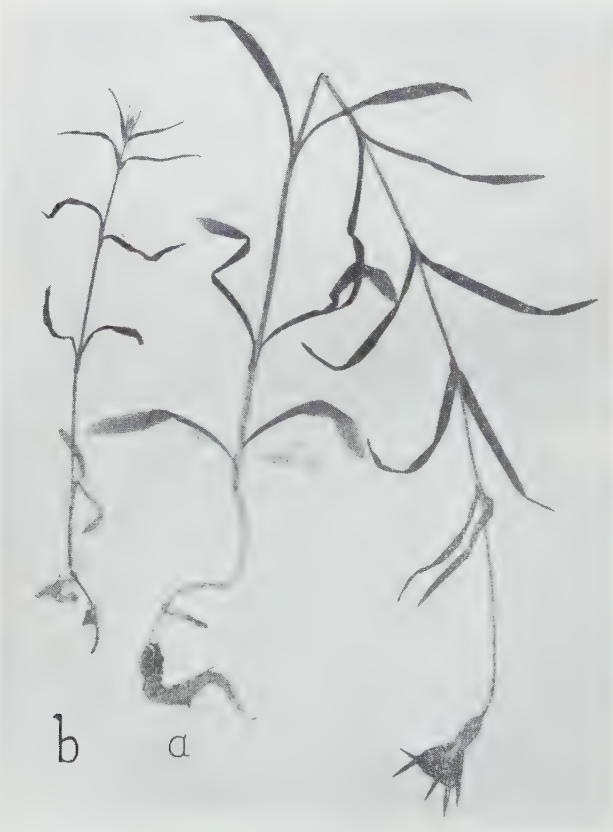

Fig. 5

Das heisst mit andern Worten, dass unsere Hemmstoffe nicht nur einen retardierenden sondern auch einen formativen Einfluss ausüben. [1])

Und ein solcher formativen Einfluss wird nicht nur im physiologischen Experiment, sondern sicher auch unter natürlichen Verhältnissen existieren. Denn wenn es auch wahr ist, dass Hemmstoffe vor allem in den Stadien des latenten Lebens, deren chemisches Charakteristikum sie ja geradezu darstellen, zu finden sind, so wurden sie andererseits auch in wachsenden Pflanzenteilen nachgewiesen (Larsen, 1939 und 1947; Linser, 1940; Pohl, 1951; Ruge, 1939).

Daher muss als sicher angenommen werden, das sie, ebenso wie die Wuchsstoffe, an der Façonierung des Pflanzenkörpers teilnehmen. Spätere Forschung wird nachzuweisen haben, ob und welche Hemmstoffe in Wuchsstoffe übergehen können und umgekehrt und welche Instanz darüber entscheidet wann, wo und unter welchen Bedingungen eine solche Umwandlung stattzufinden hat.

ZUSAMMENFASSUNG

Die Kultur verschiedener Pflanzen unter dauernder Hemmstoffwirkung zeitigte:
1. Verlangsamung des Wachstums.
2. Verkleinerung der Organe bis zur Ausbildung von Zwergformen.
3. Zurückbleiben in der Entwicklung.

Damit ist eine Methode gegeben, Pflanzen mittels ihrer eigenen Hemmstoffe die Zwergform aufzudrücken.

Praktische Anwendungen in Agri- und Hortikultur erscheinen möglich.

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[1]) Es sei hier betont, dass wir es bei unsern Hemmstoffen — im Gegensatz zu dem vielfach ähnliche Wirkungen hervorrufenden Malein Hydrazide — mit genuinen, körpereigenen Stoffen zu tun haben.

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WATER UPTAKE FROM WATER AND SALT SOLUTIONS

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1. Introduction

The process of water uptake has been investigated very extensively. Especially the work of Renner (1912, 1915, 1929) and Kramer (compiled in 1949) has delivered important data on this phenomenon. Quite recently another review of the literature appeared (Kramer 1956).

Since the investigations of Renner a difference is made between an active and a passive water uptake. At low transpiration rates an effective pressure (root pressure) in the xylem vessels is found, which manifests itself as guttation of the intact plant or as bleeding if the xylem vessels are cut. This root pressure occurs only if a vital intact root system is placed in a well-aerated medium. Various explanations have been given for this phenomenon. At the Botanical Laboratory at Groningen the exudation process has been investigated intensively (van Nie, Helder and Arisz (1950); Arisz, Helder and van Nie (1951); van Andel (1952); van Andel (1953); Arisz (1956). There are good reasons to assume, that an active transport of salt or a concentrated salt solution represents the primary part of the process causing a secondary transport of water as a consequence of the osmotic suction of the sap in the xylem vessels. Processes, similar to those involved in the exudation process, can be expected to bring about the root pressure in intact plants. It is still a matter of dispute whether the osmotic water uptake is of any importance for the total water uptake. Most authors do not ascribe an important role to the root pressure in the water transport of the plant. Kramer (1939) showed that the exudation rate of tomato and sunflower plants amounted to only 1 or 5 % of the transpiration rate. Consequently he assumes that during moderate or high transpiration rates the passive water uptake (i.e. the water uptake induced by shoot transpiration) has such a high value that the solution in the xylem vessels of the root is very diluted so that the osmotic component of the water transport is negligeable. This conclusion is based on the presumption that the exudation rates in intact plants and decapitated plants are the same. As yet this assumption is only a hypothesis.

Rufelt (1956) assumed that the osmotic water uptake and the passive water uptake take place along different tracks, so that they

are more or less independent of each other. He found in his experiments with wheat plants, that the transpiration could be diminished by applying either an osmotic counter suction in the root medium (mannitol) or by using sodiumdiethyldithiocarbamate (dieca) as an inhibitor. The decrease in transpiration was ascribed to an elimination of the osmotic water uptake. However, it is equally possible that in his experiments the water conductivity of the roots was decreased. This would have had the same effect as an inhibition of the active water uptake. Similar data of KRAMER (1951, 1940) were interpreted in this way.

TAGAWA (1934) found that the transpiration was greater on a balanced salt solution (Knopp) than on a one salt solution or an isotonic sugar solution. Here again this greater water uptake may be due to an osmotic component or an increased water conductivity.

Except for the indications given above we cannot find many data in literature in favour of the idea that the osmotic water uptake plays an important role in the total water uptake of transpiring plants. It seems desirable, therefore, to get more experimental data about the significance of the osmotic water uptake. These data were obtained using the following phenomenon.

When a root system of an intact transpiring plant is transferred from distilled water to an osmotically active solution e.g. a salt solution, then the water uptake is reduced instantaneously after the transference. Thereafter the water uptake gradually increases until after some time a new constant level is reached. These experiments have been described by BRIEGER (1928), RENNER (1929) and PERIS (1936). The decrease of the water uptake immediately after transference is caused by the osmotic counter suction of the salt solution. The following gradual increase of the water uptake was ascribed to a gradual increase of the suction tension in the xylem vessels (BRIEGER, 1928). The increase of the suction tension mentioned may be a result of:

a. a transport of ions from the medium into the xylem vessels (osmotic suction);
b. an increase of the mechanical suction, or
c. a combination of a and b.

Peris assumed that factor *a* is the most important one. However, data supporting this assumption are still lacking, for nothing is known about the ion concentration of the sap in the xylem vessels in these experiments. The experiments described below give more informations as to the causal factors involved in these transference reactions.

2. METHODS

A. *The apparatus*

The apparatus used in these experiments was described in detail elsewhere (BROUWER, 1953). The bulk of the roots is placed in the main vessel (fig. 1 A) whereas one root (reference root) is enclosed in two small potometers of 5 cm length each. As these experiments require a constant temperature, the whole apparatus is placed in a

thermostat of 40 litres capacity with heating element and stirring mechanism. This set up renders it possible to change the solution in the main vessel independently from the contents of the micropotometers. The water uptake from the main vessel and the micropotometers can be determined separately.

B. *Plant material*

The experiments have been performed with 3-5 week old bean plants (*Phaseolus multiflorus*), which were grown on a Hoagland nutrient solution.

C. *Calculations*

BRIEGER (1928) showed that the water uptake can be expressed by the formula: $U = k(S_{xylem}\text{-}S_{medium})$. In distilled water the suction tension of the medium (S_m) is zero, whereas on a salt solution the value of the osmotic suction of the medium has to be used. At the very moment of transference from distilled water to a potassium nitrate solution we get:

$$\begin{aligned} U_{d.w} &= k \times S_x \\ U_{KNO_3} &= k \times S_x - k \times S_{KNO_3} \\ \hline U_{d.w} - U_{KNO_3} &= k \times S_{KNO_3} \end{aligned}$$

In this equation S_{KNO_3} is known and $U_{d.w}$ and U_{KNO_3} are measured. So we can compute k and S_x. It appears from the course of the water uptake on potassium nitrate that U_{KNO_3} gradually increases, probably as a consequence of a change in S_x and perhaps k. The calculation given above, therefore, only applies if the uptake values immediately before and after the transference are used. However, the transference itself takes some time. To get the right uptake values we have made an extrapolation to time zero (fig. 1, 2 and 3 dotted lines). It is clear that some inaccuracy is unavoidable. If after some time the water uptake on potassium nitrate has become constant, this solution in the main vessel is changed for distilled water. Now we see immediately after the transference a highly increased water uptake, which rapidly decreases until after some time a new constant level is reached. At this transference we can use the formulae:

$$\begin{aligned} U'_{d.w} &= k' \times S'_x \\ U'_{KNO_3} &= k' \times S'_x - k' \times S_{KNO_3} \\ \hline U'_{d.w} - U'_{KNO_3} &= k' \times S_{KNO_3} \end{aligned}$$

From the latter equation we can compute S_x and k'.

It appears that S'_x has a much higher value than S_x, whereas in many occasions k' is greater than k (See ARISZ, 1956 page 37).

From Dixon's cohaesion theory we know, that a loss of water from the leaves gives rise to a mechanical suction tension in the xylem vessels not only of the leaves but also of the root. We may expect that on distilled water the suction tension in the xylem vessels represents a mainly mechanic suction. If, however, the root system is placed on

a salt solution, a transport of a salt solution into the xylem vessels may be expected and as a consequence of such a salt transport an osmotic suction may occur. The formula of Brieger runs into: $U = k\ (S_{mech} + S_{osm}) - S_{med}$. During the steady state on distilled water the water uptake by the roots will be equal to the water loss by the shoot and we may expect, therefore, a constant S_{mech}, whereas the S_{osm} probably will be about zero. Immediately after transference from distilled water to a salt solution the loss of water due to transpiration is much greater than the reduced water uptake (Arisz, 1956, p. 35-37). This results in an increase of the S_{mech}. Furthermore we expect now an increase of the S_{osm} due to a permeation of salt ions into the xylem vessels. The increase of S_x to S'_x, therefore, can be a consequence of the mechanical component and/or an increase of the osmotic component of the suction tension. We have to consider the following possibilities:

$$S'_x > S_x \qquad \begin{array}{ll} 1 & S'_{x\,mech} = S_{x\,mech};\ S'_{x\,osm} > S_{x\,osm} \\ 2 & S'_{x\,mech} > S_{x\,mech};\ S'_{x\,osm} = S_{x\,osm} \\ 3 & S'_{x\,mech} > S_{x\,mech};\ S'_{x\,osm} > S_{x\,osm} \end{array}$$

To decide which of these three possibilities is realised we started from the following line of thought. If there is distilled water in the main vessel and in the micropotometers a mechanic suction tension will occur in the xylem vessels which is the same for main roots and reference root. After placing the main roots from distilled water in a salt solution an increase of the mechanic suction tension will result in an increasing water uptake both by the main roots and the reference root. The occurrence of an osmotic suction will be restricted to those parts of the root system, which are in contact with the salt solution, and will not influence the water uptake of the reference root. A comparison of the course of the water uptake by main roots and reference root will give an insight into the nature of the increased suction tension.

3. Experimental results

a. *The transference reactions*

The experimental set up and the results of a typical transference experiment are shown in figure 1. After a period of constant water uptake on distilled (demineralized) water present in main vessel and micro potometers a 75 mM calcium chloride solution was brought into the main vessel. From the leap in the water uptake values we computed an S_x of 2.4 atm and a k of 26 mm³/3 min 1 atm. After some time on calcium chloride the water uptake reached a new constant level. Now we changed again the solution in the main vessel and substituted the calcium chloride solution by distilled water. The value of the water uptake from the main vessel shows the normal picture as described in literature. From this leap we computed an S'_x of 5.5 atm and a k' of 27 mm³/3 min 1 atm. We see from these values that the

suction tension showed an important increase whereas the conductivity did not change.

If we trace the water uptake by the reference root (fig. 1C) we see that this uptake also showed an important increase after transference of the main roots to the calcium chloride solution and a subsequent decrease after transference back to distilled water. At first sight the

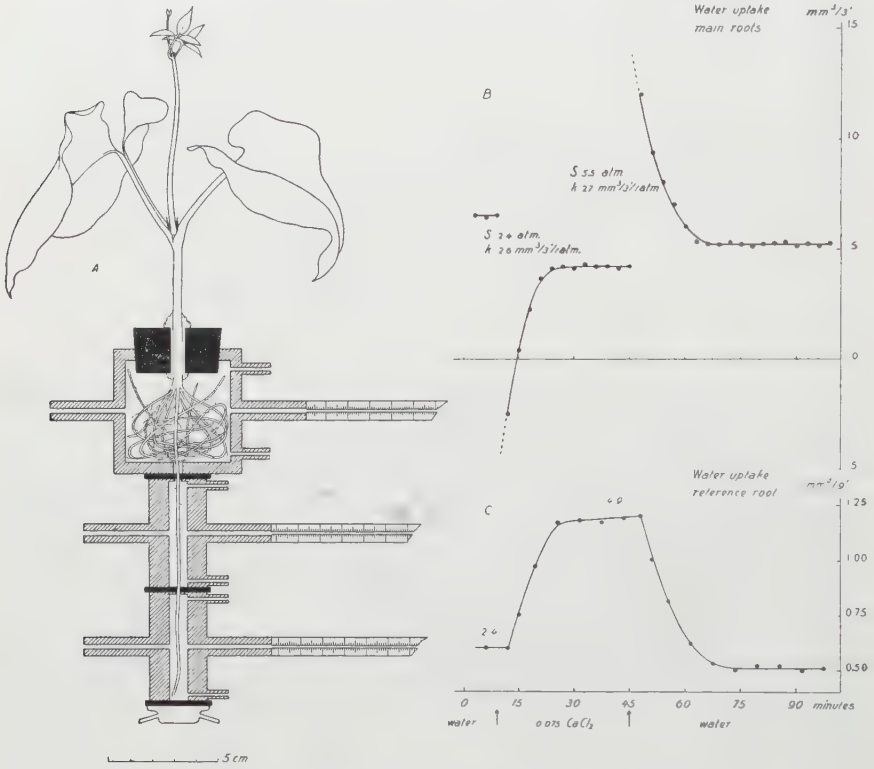

Fig. 1. A. The experimental set-up. A bean plant placed on a perspex vessel, the main roots in a large vessel (main vessel) and the reference root in two micropotometers; B. The course of the water uptake by the main roots at transference from distilled water to a salt solution and at transference from the salt solution back to distilled water; C. The course of the water uptake by the reference root on distilled water at the transference of the main roots to a salt solution and back to distilled water

course of the water uptake by main roots and reference root is identical. Starting from the supposition that the mechanical suction is equal over the whole root system we may assume that the suction tension in the reference root amounted to 2.4 atm at the beginning of the experiment. Further it seems likely that after transference of the main roots to calcium chloride a migration of ions into the xylem vessels of the reference root did not occur. Thus, the water uptake by the reference root can be used as a measure for the mechanical suction

tension. This is the more likely because the conductivity of the root system remained unchanged. In this way we find a mechanical suction tension of 4.9 atm. The total suction tension in the main roots amounted to 5.5 atm (see above). The difference between total suction tension and mechanical suction tension (5.5 — 4.9 = 0.6 atm) represents the osmotical component of the suction tension in the main roots.

From these values we may conclude that the increase of the water uptake after transference to a calcium chloride solution by far the greatest part is caused by an increase of the mechanical component of the suction tension. The osmotic component of the suction tension causing the osmotic water uptake amounted to only a 10 %, even on such a concentrated salt solution. The assumption of Peris (1936) that the whole phenomenon was due to a permeation of ions into the root must be discarded.

b. *Comparison of different concentrations of the same salt*

Figure 2 gives the course of the water uptake at transference from distilled water to a 5.0 atm potassium nitrate solution and to a 2.5 atm

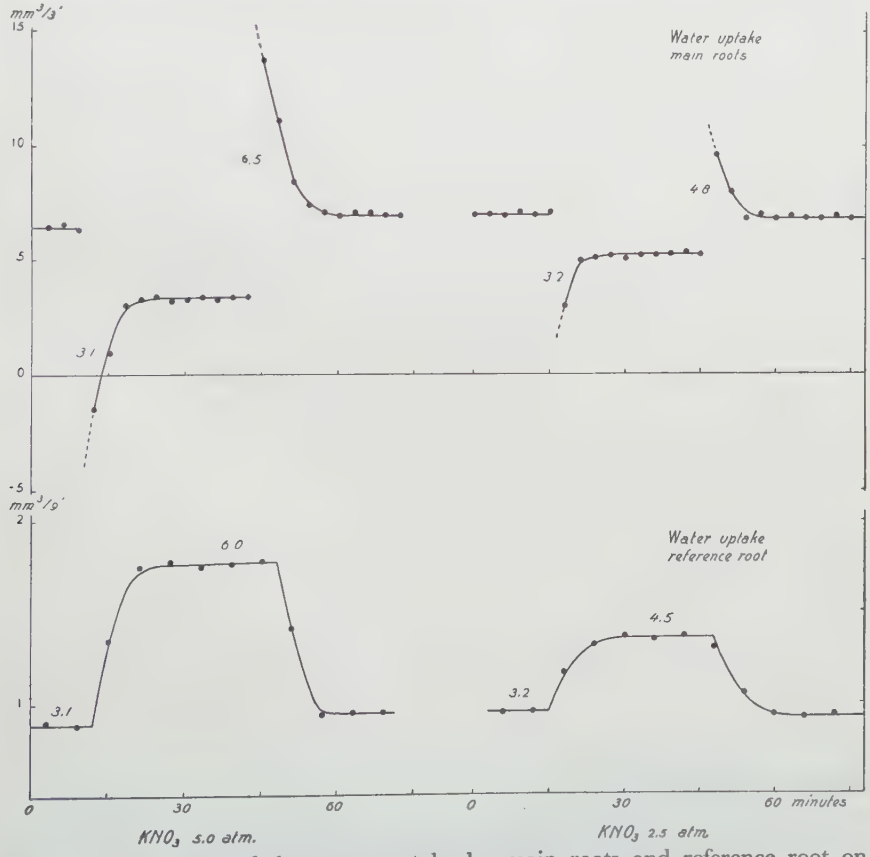

Fig. 2. The course of the water uptake by main roots and reference root on distilled water, and after transference of the main roots to a salt solution and back to distilled water

potassium nitrate solution and back to distilled water. On 5.0 atm potassium nitrate the osmotic component of the suction tension amounted to 6.5 — 6.0 = 0.5 atm and on 2.5 atm potassium nitrate to 4.8 — 4.5 = 0.3 atm. The osmotic component is higher at the more concentrated solution. It is essential that such experiments are performed with the same plant in time periods as short as possible. This is self evident because different plants show different transpiration rates. The osmotic component is most probably an indication of the salt concentration in the xylem vessels. The total salt transport, therefore, amounts to water uptake times concentration. The water uptake on the more concentrated solution is lower than on the more diluted one. The product water uptake times concentration is about the same in both cases. Many experiments with different salts, performed in this way, have given the same picture.

c. *Comparison of the same concentration of different salts*

The results of a comparison of potassium chloride with potassium nitrate (4.2 atm) are plotted in figure 3. On potassium chloride we see an increase of the suction tension from 2.8 to 5.8 atm, whereas the mechanical component increased from 2.8 to 5.6 atm. The osmotic component amounts here to 0.2 atm. With the same plant the osmotic component on potassium nitrate amounted to 0.4 atm i.e about twice as great as on potassium chloride. The water uptake values are about

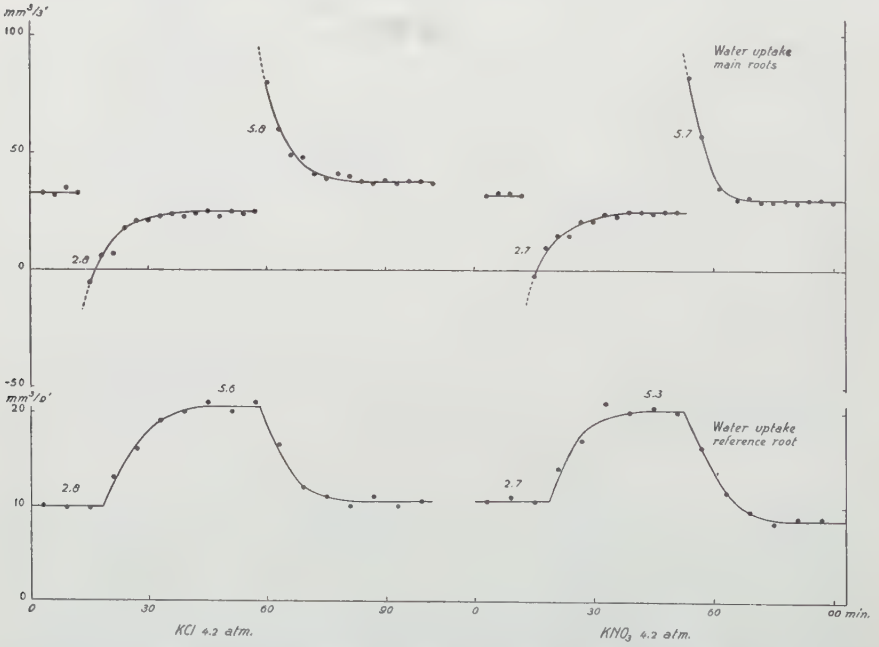

Fig. 3. The course of the water uptake by main roots and reference root on distilled water, after transference of the main roots to a salt solution and after transference back to distilled water.

the same in both cases. So the salt uptake is greater in the latter case. With all plants this appeared to be true. The mean ratio: $\frac{KNO_3}{KCl}$ amounted to 1.82.

By comparing in the same way potassium chloride with calcium chloride it appeared that the osmotic component was greatest on potassium chloride.

4. Discussion

From the experiments described above, it is evident that under the given experimental circumstances the water uptake is mainly determined by a mechanic suction tension in the xylem vessels of the root. Even on such concentrated salt solutions the osmotic component amounts to only 4-10 %.

These results are in agreement with the supposition given by Kramer. In view of the experiments of Rufelt (1956) with wheat plants, it may be that a generalisation of this principle is not allowed.

The question arises in how far a lack of aeration during the application of the salt solutions (30-45 minutes) inhibited the development of a higher osmotic component. At the beginning of the application the solutions were air-saturated, whereas after 45 minutes about 40 % of the oxygen was utilised. We can not speak, therefore, of a large lack of oxygen. Comparative experiments with this material showed that the transpiration on salt solutions with and without aeration did not differ very much, this in contrast with barley plants. The difference was of the same order as the osmotic component calculated from the experiments described above. An example of such an experiment is given in figure 4. However, very soon after stopping the aeration and the subsequent decrease of transpiration the transpiration showed

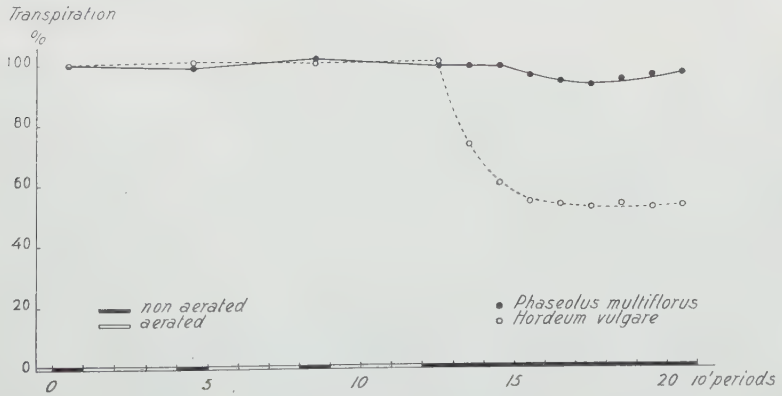

Fig. 4. Comparison of the transpiration rates on a 2.5 atm potassium nitrate solution with and without aeration. The transpiration was determined by weighing during 10 minutes periods. Aerated means alternating 10′ without aeration (weighing) and 30′ with aeration. Non-aerated means without aeration throughout the whole period

an increase regulated by the shoot's suction. However, the osmotic component seems to be a real thing.

Furtheron the experiments showed that the mechanic water uptake, as occurring on distilled water, can be reduced by an osmotic counter suction in the medium. This osmotic counter suction reduces the water uptake immediately to very low values. It is true that we get after transference a gradually increasing water uptake but the subsequent steady state level is lower than the level on distilled water as is the transpiration (see ARISZ 1956 fig. 27). These observations are contrary to the supposition of Rufelt that the mechanical water uptake can not be inhibited by an osmotic agent in the medium because the mechanic uptake takes place via non-semipermeable root parts. If this assumption is correct, the water uptake can not be expected to decrease when the plant is transferred from distilled water to a salt solution.

The author is grateful to Professor Dr. W. H. Arisz for suggesting the problem and valuable discussions of the results.

SUMMARY

The aim of the investigation was an analysis of the transference reactions (Überführungsreaktionen).

These reactions were studied with bean plants.

At transference of the root system of an intact transpiring plant from distilled water to a salt solution the water uptake decreases instantaneously. Thereafter a gradual increase is found until a new constant level is reached. At transference from a salt solution to distilled water the water uptake increases and shows a gradual decrease to a constant level.

It is shown that this course of the water uptake by far the greatest part is regulated by the mechanical suction tension in the xylem vessels and that only a small fraction of the water uptake is due to osmotic forces.

The experimental set-up rendered it possible to make a separation of these two mechanisms.

It appeared to be possible to inhibit the nearly fully mechanic water uptake by means of an osmotic counter suction in the medium.

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ACTA BOTANICA NEERLANDICA, Vol. 5 (3), 1956

NOTES ON MYRTACEAE. VI

BY

G. JANE H. AMSHOFF

(*received August 8th, 1956*)

Psidium Molinae Amsh. n. sp.; — *Eugenia Molinae* Standley in sched. Frutex ramulis novellis dense breviter brunneo-pubescentibus. Folia parva, ovato-oblonga, obtusa, 1½-2½ cm (interdum usque ad 4 cm) longa, 9-12 mm lata, glabra. Pedicelli graciles, glabri, 1-1½ cm longi. Alabastra circ. 4 mm longa, glabra, apice minute aperta, brevissime 4-loba. Calyx intus pubescens, in flore adulto irregulariter 4-fissus. Petala 4 mm longa. Antherae orbiculares. Stylus glaber; stigma vix dilatatum. Ovarium biloculare, pauci-ovulatum, ovulis uniseriatis, in placenta bilamellata affixis. Bacca parva, in sicco vix 1 cm in diametro, segmentis calycis persistentibus coronata; semina circ. 3.

Honduras: Dept. Morazan, hills near Zamoran, Rodriguez 232 *fr.*, Standley 21255 *fl.*, type, id. 11777 et 4080.

Near Camino San Antonio, Rodriguez 1388; slopes of Cerro de Uyuca, Standley 26238 *fl.*

Dept. Comayagua, vicinity of Comayagua, Standley and Cacon 5393.

Dept. El Paraiso, in Quebrada Dantas, 5 km east of Oyo de Agua, alt. circ. 700 m, Williams 11584, 12817.

Quebrada El Maro, 20 km north of Yuscaran, Molina 1670 *ster.*

Las Casitas, alt. 950 m, Standley, Williams and Allen 570, Williams and Molina 14143 (MO) *fl.*, distributed under the name of *Eugenia Molinae* Standley. In Quebrada along creek near Casitas, Williams and Molina 11536 *fl.*

Dept. Olancho, between Juticalpa and El Aliso, alt. 380–400 m, Standley 17757 (all in F).

Nearly all specimens were originally identified as *P. Sartorianum* (Berg) Ndz., a species which is at once distinguishable from our new one by its calyptrate calyx and shortly acuminate leaves. On account of this calyptrate calyx *P. Sartorianum* and its nearest allies were placed by BURRET in Notizblatt Vol. XV.3 (1941) in a new genus, *Mitropsidium* Burret but on account of the structure of the calyx *P. Molinae* should, on the contrary, be included in the group of species which Burret left in the genus *Psidium* L., for in these species the calyx is either open or closed at the apex and splits at flowering-time more or less regularly into 4 or 5 segments. Yet this character of the calyx has to be used with circumspection as e.g. in *Psidium* (*Calyptropsidium*) *Friedrichsthalianum* (Berg) Ndz. the calyx is sometimes calyptrate and sometimes vertically dehiscent (with, however, the two or three segments still circumcissile at the base); and a specimen collected by Makrinius in the Mexican state of Oaxaca (sub nr. 609) has the flowers of *P. Molinae* and the leaves of *P. Sartorianum*. The material collected in Honduras, however, is so abundant and so uniform that it seems

advisable to recognize it as a distinct species, for which I have accepted Standley's epithet *Molinae*.

Other Central American species nearly allied to *P. Sartorianum*, and perhaps merely representing forms of this species, are *P. yucatanense* Lundell, from the Yucatan Peninsula of Mexico and Br. Honduras, which has more distinctly acuminate, very acute leaves, an indumentum consisting of shorter, straight hairs on the young twigs, and a less neatly dehiscent calyx, and *P. Solisii* Standley, which has already been described in the previous century under the name of *Calyptranthes Tonduzii* Donn. Smith, and which is provided with obtuse leaves; the latter occurs in Costa Rica and should on present evidence be included in *P. Sartorianum*.

Eugenia nicaraguensis Amsh. n. sp.

Frutex vel arbuscula ramulis tomentosis. Folia obovata, basi acuta, apice rotundata vel rarius obtusa vel emarginata, membranacea, 8–10 cm longa, 4–6 cm lata, supra fere glabra, subtus pilis dibranchiatis tortuosis dense pubescentia; nervis lateralibus utrinque 4–6, arcuato-ascendentibus, utrinque prominulis. Petiolus circ. 7 mm longus. Pedicelli solitarii vel bini, 1–3 cm longi, axillares vel infra-axillares, pilis dibranchiatis pubescentes. Sepala suborbicularia, pubescentia, circ. 5 mm longa. Petala oblonga, circ. 1 cm longa. Ovarium pubescens, 2-loculare, loculis parvis, 10- usque ad 5-ovulatis, ovulis in placenta peltata affixis. Fructus ignotus.

Nicaragua: Dept. Chontales, vicinity of Juigalpa, Standley 9427, type, id. 9466, 9211 and 9289 (F).

Dept. Jinotego, along trail between Jinotego and Las Meseitas, West of Jinotego, Standley 9813 (F), "petals and stamens white; very showy in flower" (Standley).

Vern. name: Guacoco.

These specimens were originally identified as *P. Rensonianum* Standley. Dissection of the fruit of the latter species (I saw the type and the cotype, both collected in El Salvador) proved that this species too belongs to the genus *Eugenia*, but by its racemose inflorescence and shorter indumentum it is more nearly allied to *E. guatamalensis* Donn. Smith and to *E. Faydenii* Krug et Urban than to *E. nicaraguensis*. Unfortunately, *P. Rensonianum* Standley is known only in two fruiting specimens; the flowers are not known, and for that reason its exact position in the genus *Eugenia* cannot yet be ascertained.

In his "Flora of Costa Rica" Standley cites *Psidium Rensonianum* as belonging to the Costa Rican flora. The following specimens cited from Costa Rica have been inspected by me:

San José, Wercklé 16437 *fr.*; El Rodeo, Villa Colon, Valerio 944 *fr.*; San Pedro de San Ramon, Orillas del Rio Barranca, Brenez 21873; Entre Canaz y Filaran, Guanacaste, Brenez 12688 *fl.*; Woods near the bridge of the Rio Grande, Pacific railway, Pittier herb. Costaric. 16405.

These Costa Rican specimens belong to an *Eugenia* species. They differ from *P. Rensonianum* by the much larger leaves (up to 16 cm long and 16 cm wide) and by the thicker lateral nerves, and from

E. nicaraguensis Amsh. by the few-flowered racemes, smaller flowers and again by the larger leaves. On present evidence it is quite likely that they belong to *E. hiraeifolia* Standley, a Panama species of which the flowers have not yet been collected in Panama; its fruits, however, are perhaps somewhat larger than in the specimens from Costa Rica. *E. hiraeifolia* Standley, if this is indeed the correct name, had already been collected in Costa Rica by Oerstedt (ad Aqualacante, Oerstedt 4014), but his specimen was so imperfect that the monographer Berg, who studied the *Myrtaceae* of the collection Oerstedt, refrained from describing it.

The species has been collected in Guatemala also (Dept. Guatemala, Aguilar 137 *fr.*).

Acrandra guianensis Amsh. n. sp.

Arbor parva vel frutex? Folia ovata, petiolata, herbacea, basi obtusa vel rotundata, apice acute acuminata, circ. 10 cm longa et 4-6 cm lata, supra sparse, subtus dense pubescentia; nervi laterales circ. 12, supra paullulum impressi, subtus prominentes, arcuato-anastomosantes. Flores fasciculati, plerumque laterales; pedicelli circ. 5 mm longi, apice bibracteolati. Flores 4-meri. Sepala in alabastro imbricata, inferiora petala tegentia, in flore aperto reflexa, circ. 6 mm longa, extus pubescentia, intus glabra. Petala oblonga, circ. 9 mm longa, ciliata. Stamina numerosa; antherae subbasifixae; connectivo apice caudatim producto. Ovarium pubescens, 2-loculare: ovulis circ. 8 pro loculo. Fructus ignotus.

Br. Guiana: Tumaturi, in dense upland forest, Gleason 446 *fl.* (NY).

This is the first representative of the genus *Acrandra* collected outside Brazil. On account of the 4-merous flowers and 2-celled ovary, and in the absence of ripe fruits, I have long hesitated before inserting this species in the genus *Acrandra* Berg, to which the structure of the anthers undoubtedly points. However, as in the allied genera *Myrtus* and *Ugni* the flowers may be either 4- or 5-merous, and the ovary often is but few-celled, the fact that in the few species of *Acrandra* that hitherto have been described, the ovary is 7- to 9-celled and the flowers are 5-merous, cannot be regarded as a serious obstacle. In most respects the plant described here as *Acrandra guianensis* suggests *Eugenia*, but in the very characteristic structure of the anthers it resembles *Acrandra*. If my surmise is correct, it is to be expected that the seeds and the embryo of this new species will prove to be of the kind found in *Psidium*.

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THE IMPORTANCE OF AMINO ACIDS FOR THE DEVELOPMENT OF FUSARIUM OXYSPORUM F. LUPINI SN. ET H. IN THE XYLEM OF LUPINS

BY

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Laboratory of Phytopathology, Wageningen

Mededeling 166

(*received September 8th, 1956*)

Introduction

Fusarium oxysporum f. *lupini* is the cause of the wilting disease of lupins. The fungus develops primarily in the xylem vessels of roots and stem. Since the xylem sap of herbaceous plants usually does not contain sugars or other organic substances (Kramer, 1949), the question arises from where the fungus derives its organic substrates. This problem may be of some importance for the study of the causes of the resistance which certain varieties of lupins have developed against this disease. It has been shown that development of the fungus is inhibited in resistant plants, although some growth is possible.

Zeevaart (1955) found aspartic acid in the exudation sap of *Lupinus luteus* L., and suggested that this could be used as a source of carbon; Saaltink (unpublished results) showed later that glutamic acid, asparagine, alanine and probably leucine were also present, aspartic acid being quantitatively the most important. Potapov and Cseh (1955) demonstrated the presence of several amino acids in the sap of maize and pumpkin.

It is known that *Fusarium oxysporum* f. *lycopersici* is able to grow in a medium containing no other organic substances than a single amino acid. (Gottlieb, 1946). However, the amounts Zeevaart found in exudation sap were considerably less than those used in Gottlieb's experiments.

In the experiments described below the growth of *Fusarium oxysporum* f. *lupini* was determined in solutions of different amino acids in varying concentrations. Special attention has been given to the effect of those amino acids that have been found in the exudation sap of lupins, and to the influence of the concentration of the amino acids.

Material and Method

Richards' solution containing 10 gm KNO_3, 5 gm KH_2PO_4, 2.5 gm $MgSO_4$. 7 aq, a trace $FeCl_3$ in a litre distilled water was used as a basic solution to which organic substances were added in different concentrations.

The pH of the solution was 4.1. When amino acids had been added the pH was adjusted by means of potassiumhydroxyde.

The fungus was grown in 100 ml erlemeyers containing 25 ml of the solution. Larger quantities of liquid were put in proportionally larger erlemeyers in order to maintain a more or less constant proportion between volume and surface. The solutions were inoculated either by means of small disks which had been punched into an agarplate which was homogeneously covered by the fungus, or by adding 0.5 ml of a spore suspension obtained from a "shaking" culture. The agar disks themselves allowed some growth, so that the controls (no organic substance present) yielded dry weights of 10–30 mg. The erlemeyers were incubated at 25° C. for 7–10 days, in undisturbed cultures, but the flasks were shaken once or twice a day. At the end of the incubation period the mycelia were filtered and washed with dilute nitric acid. They were then washed several times with distilled water, dried and weighed. The nitric acid was used to remove a precipitate that was formed in the media when the pH rose above 7. The pH of the filtrate was determined. The experiments were carried out in duplicate or triplicate. The tables and figures give the average values.

The fungi that were used for the experiments comprised several strains of *Fusarium oxysporum* f. *lupini* isolated by G. J. Saaltink, Laboratory of Phytopathology, Wageningen.

Several experiments were repeated with a strain of *Fusarium oxysporum* f. *callistephi*. The results were the same.

Results and discussion

F. oxysporum f. *lupini* proved to be able to grow in a medium containing 0.5 % aspartic acid, glutamic acid, ornithine, arginine, glycine or alanine. The yield of dry weight decreased from aspartic acid to alanine in the order mentioned above. The former two amino acids allowed an even better growth than 0.5 % glucose controls. In solutions of leucine, valine and phenylalanine, however, dry weights were found which surpassed those of the controls (i.e. solutions lacking organic substances) only by a few milligrams.

An identical series of experiments was carried out with 1 % glucose added to the solutions. None of the added amino acids had a toxic effect; all of them, even leucine and valine promoted growth as compared with the control solutions containing glucose only (both 1 and 1.5 %).

It is of little use to compare the nutritional value of the amino acids and of glucose or sucrose, because the added amounts are difficult to compare. In these experiments we added 0.5 % organic substances as did Gottlieb (1946) in his investigations. This means, however, that there are great differences in concentration. As will be shown later, the concentration of amino acid is very important for its utilization. In another experiment 0.035 M of every amino acid was added. In this case arginine proved to be a better substrate than

aspartic acid, but growth on glucose and sucrose alone was about twice as great as on the arginine.

The importance for growth of aspartic acid, glutamic acid, asparagine, alanine and leucine was examined in greater detail.

Fig. 1 shows the relation between growth of the fungus and the concentration of aspartic acid in the medium. 0.075 M. aspartic acid appeared to yield maximal growth; a further increase in the concentration of aspartic acid resulted in a reduction of the dry weight; in some cases it was reduced almost to zero. Growth did not start at concentrations below 0.0125 M.

When the medium contained both glucose and aspartic acid the relation between growth and amino acid concentration was more or less linear. The same results were obtained when nitrate was omitted

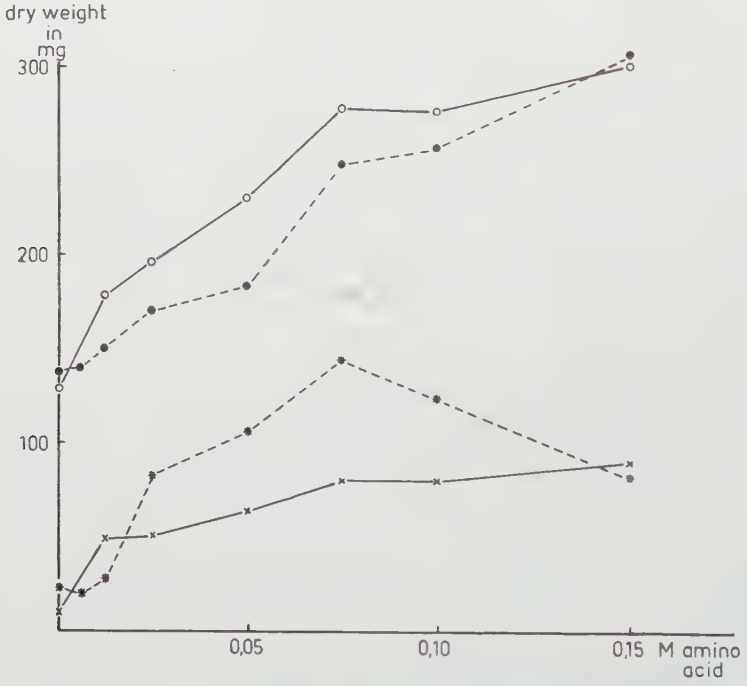

Fig. 1. Relation between growth and amino acid concentration of the medium. *------* aspartic acid; ●------● aspartic acid and 0.05 M glucose; ×———× glutamic acid; ○———○ glutamic acid and 0.05 M glucose.

from the solution. It is probable therefore that aspartic acid is being used as a source of both carbon and nitrogen.

The pH of the medium increased, especially when no glucose was present.

Glutamic acid gave somewhat different results (Fig. 1). Growth began to increase with increasing concentration. Increasing the concentration above 0.075 M, however, did not result in any further

increase in dry weight production. This held true both for solutions with and without glucose. Addition of more glucose did, however, increase the growth. Apparently some factor is lacking which is specifically necessary for the utilization of glutamic acid. We did not succeed in removing this restriction by adding trace elements, especially zinc and iron, which appeared to be very important when glucose was used as source of carbon. A solution of vitamins containing riboflavin, nicotinic acid amide, p-aminobenzoic acid, pyridoxine, aneurine and biotin or diluted expressed sap of lupins did not have any effect either.

Asparagine proved to be a rather unsuitable substrate (Table 1). Larger amounts of asparagine promoted growth, but only if at least

TABLE 1

The effect of asparagine on the dry weight production in mg.

Asparagine \ Glucose	0.00 M	0.10 M	0.25 M
0.150 M	31.7	93.7	273.6
0.100 „	31.2	128.1	257.4
0.075 „	23.5	119.7	169.0
0.050 „	22.3	127.1	128.7
0.025 „	36.1	120.6	135.0
0.013 „	25.1	106.3	126.1
0.000 „	18.2	98.1	101.9

0.25 M glucose was present in the medium. It is to be questioned whether this increase in growth can be ascribed to the effect of the asparagine itself. It might be possible that the asparagine is acting as a source of nitrogen, although on account of former experiments the amount of potassium nitrate present is supposed to be sufficient. The presence of trace elements in the asparagine is another possible explanation. The growth in a glucose medium is very sensitive to the addition of trace elements; this has been mentioned before.

In Table 2 it is shown that alanine and leucine were not very good sources of carbon either.

TABLE 2

Leucine and alanine as a source of carbon for *Fusarium oxysporum* f. *lupini*. Dry weights in mg.

Leucine \ Glucose	0.00 M	0.20 M	Alanine \ Glucose	0.00 M	0.20 M
0.075 M	52.7	135.3	0.075 M	14.4	203.0
0.050 „	18.7	138.4	0.050 „	4.5	197.3
0.025 „	11.6	72.3	0.025 „	0.0	169.3
0.013 „	2.5	93.6	0.013 „	0.0	157.2
0.006 „	0.0	69.5	0.006 „	0.0	174.0
0.000 „	0.0	98.6	0.000 „	0.0	155.7

It is known that mixtures of amino acids often constitute a better substrate than solutions containing a single amino acid. The fungus grew very well in a medium containing Difco Bacto casaminoacids as is demonstrated in Table 3.

There was never any inhibition of growth, but an increase in concentration above 4.3 % increased the dry weight only when the medium contained more than 0.05 M glucose. This is the same phenomenon we were confronted with in the experiment described in Table 1.

TABLE 3

Effect of casaminoacids on growth in the presence and absence of glucose. Dry weights in mg.

Glycose / Casaminoacids	0.00 M	0.05 M	0.20 M
6.30 %	172.7	223.8	429.5
4.73 „	186.6	237.1	319.8
3.15 „	151.4	136.8	200.6
1.58 „	110.5	76.0	161.3
0.79 „	64.3	100.2	191.1
0.39 „	35.3	93.8	181.2
0.00 „	26.3	54.8	142.1

These solutions contain 0.10; 0.075 etc. M. glutamic acid.

Mixtures of two or more amino acids in varying proportions (aspartic acid/glutamic acid; asparagine/glutamic acid; aspartic acid/glutamic acid; asparagine/alanine/leucine; etc.) yielded higher dry weights than any one of the components alone. A solution containing 0.050 M glutamic acid 0.0015 M aspartic acid, 0.0024 M leucine and 0.0020 M alanine produced 89.1 mg dry matter. However a solution of 0.05 M glutamic acid alone produced only 31.8 mg., and a 0.10 M solution of glutamic acid 59.7 mg. The same solutions diluted four times (0.0125 M. glutamic acid, etc.) did not allow any growth at all.

Thus we have found, in agreement with the results of Anderson and Emmart (1934) and Gottlieb (1946) with *Fusarium oxysporum* f. *lycopersici*, that *Fusarium oxysporum* f. *lupini* is capable of using various amino acids as its only source of carbon. The most favourable concentrations were found between 0.050 and 0.075 M. There was relatively little increase in growth at higher concentrations. We must conclude that under our experimental conditions some factor essential for the utilization of some amino acids seems to be lacking.

From the figures and tables presented, it can be seen that perceptible amounts of mycelium are usually found at concentrations of 0.0125 M. and higher. Zeevaart (1949) estimated the aspartic acid concentration of exudation sap of non-inoculated lupins at about 0.02 % or 0.0015 M. In the sap of inoculated plants the concentration was still less. It is probable that the liquid in the xylem vessels of intact transpiring plants is even more dilute. On the other hand amino

acids may continuously be given off to the vessels so that the concentration of the sap is being kept more or less constant, while in our experiments the concentration is gradually decreasing.

Table 4 shows the results of an experiment in which 100, 50, 25 and 12.5 ml of different solutions of aspartic acid were inoculated with the same amount of fungus. The lowest concentration — 0.0125 M. — did not allow any growth in the flasks containing 12.5 ml., but 50 ml yielded some mycelium, and 100 ml. allowed good growth.

TABLE 4

Effect of quantity and concentration on the utilization of aspartic acid

	100.0 ml	50.0 ml	25.0 ml	12.5 ml
0.050 M	448.3	216.7	170.2	72.1
0.025 „	319.8	193.3	131.9	64.8
0.013 „	112.9	76.2	51.0	42.2
0.000 „			40.5	

However, doubling the concentration has a much greater effect than doubling the quantity when low concentrations are used.

In another experiment 50 mg of aspartic acid was added to erlemeyers respectively containing 100, 50, 25 and 12,5 ml of Richards' solution. The amino acid concentration ranged from 0.05 to 0.4 %. The first and second solution yielded dry weights of 33.2 and 32 mg, the third 44.2 mg and the fourth — the highest concentration — 84 mg. This shows very clearly that very low concentrations cannot be compensated for by greater quantities of the solution. A solution containing less than 0.1 % aspartic acid is apparently too greatly diluted for the fungus to take up enough of the substance.

It therefore seems unlikely that the amino acids in the xylem sap of lupins are very important as a source of carbon for *Fusarium*. It is more probable that the fungus draws the necessary organic substances from the adjacent living cells. This conclusion is supported by the following experiment. Lupins were decapitated, and the exudation sap was collected during twenty four hours. When 25 ml of this sap was inoculated, and incubated in the usual way the yield was 3.5 mg. Dry weights of 200–300 mg were found when glucose had been added to the sap. There was no indication of the presence of an inhibiting factor, even in the sap of resistant plants, but it was merely the lack of organic substrates which seemed to prevent growth.

We may add here that SANWAL (1956) found that *F. oxysporum* f. *lycopersici* developed mycelium in a medium of aspartic or glumatic acid, although the organism was unable to produce toxins under these conditions.

SUMMARY

Fusarium oxysporum f. *lupini* has been shown to grow in media with one or more amino acids as the only source of carbon. Growth, however, was usually less than in equimolar solutions of sugar. Particular attention was given to the importance of aspartic acid, glutamic acid, asparagine, alanine and leucine. The three latter

substances proved to be rather unsuitable substrates for this fungus, although it is possible that some factor essential for their utilization is lacking in these experiments, as must be the case for glutamic acid. Aspartic acid became toxic in concentrations of about 0.1 M.

The minimal concentration allowing growth, was shown to be about 0.0125 M. The disadvantages of a low concentration could not be compensated for by greater quantities of the diluted solution.

Comparing the results with the data known about the presence and concentration of amino acids in the xylem vessels of lupins we must conclude that these substances cannot be very important for the development of *Fusarium* in the xylem vessels.

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INVESTIGATIONS INTO THE OCCURRENCE OF ACTIVE AND PASSIVE COMPONENTS IN THE ION UPTAKE BY *VICIA FABA*

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I. Introduction

In previous publications (Brouwer, 1953*b*, 1954*b*) the author described the influence of the water uptake on the ion uptake in *Vicia faba*. It was found that with stronger transpiration the ion uptake was greater than with weak transpiration. Yet water uptake and ion uptake were not absolutely coupled. From the experiments it appeared that:

a. by the use of inhibitors the ion uptake could be inhibited independent of the water uptake (fig. 1);

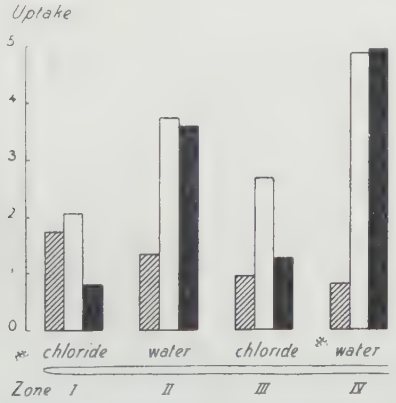

Fig. 1. Influence of 2.4-dinitrophenol, 10^{-5} M, on the water and chloride uptake at high suction tension in the xylem vessels. Chloride and water uptake of the various root zones at low suction tension (shaded blocks), at high suction tension without inhibitor (white blocks) and at high suction tension with inhibitor (black blocks). The nutrient solution contained 5 mM calcium chloride with or without inhibitor. The shoot was constantly illuminated. The suction tension in the xylem vessels of the root was increased by enhancing the osmotic value of the solution in the main vessel (vide Brouwer, 1954). With dinitrophenol in the nutrient solution the chloride uptake was reduced to about 50 % without a reduction of the water uptake. Scale unity 14 γ Cl or 200 mm^3 of water per zone per 24 hours.

b. by an osmotic counter suction in the medium the water uptake could be inhibited without influencing the ion uptake (fig. 2);

c. in an average of a large number of experiments in which either the water uptake or the ion uptake was determined by the various root zones, the ratio:

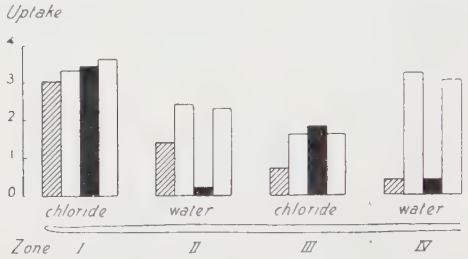

Fig. 2. Influence of an osmotic counter suction with sucrose in the nutrient solution on the water and chloride uptake at high suction tension in the xylem vessels. Chloride and water uptake of the various zones of the root at low suction tension (shaded blocks), at high suction tension without sucrose (white blocks) and at high suction tension with an osmotic counter suction (black blocks) in the medium. The sucrose solution strongly decreases the water uptake without a reduction of the chloride uptake. Scale unity 14 γ Cl or 200 mm^3 of water per zone per 24 hours.

$$\frac{\text{chloride uptake at high transpiration}}{\text{chloride uptake at low transpiration}}$$

showed more parallelism with the ratio:

$$\frac{\text{waterconductivity at high transpiration}}{\text{waterconductivity at low transpiration}}$$

than with the ratio:

$$\frac{\text{water uptake at high transpiration}}{\text{water uptake at low transpiration}}$$ (fig. 3).

These facts induced the author to state the hypothesis that the increased ion uptake, as it is found at a higher transpiration, is to be attributed to an increased conductivity for ions in the root tissue, with the turgescence of the tissue as a causal factor for the change in conductivity. The influence of the water uptake on the ion uptake therefore was considered to be of an indirect nature.

Before at the hand of experiments with *Pisum sativum* Hylmö (1953) had developed a theory, in which the influence of the transpiration on the ion uptake was called direct. He distinguished the ion uptake into three components, of which one (phase I) was independent of the water uptake (diffusion and absorption). The accumulation in the root (phase II) appeared to be dependent on the water uptake as far as the chloride uptake was concerned. Finally a third component (phase III) was left, which increased directly proportional to the water uptake and at a water uptake nihil appeared to be practically nihil too. It is this third phase, the salt transport to the shoot, which in Hylmö's experiments strongly determined the whole relation between ion uptake and water uptake, because diffusion and

accumulation in the root tissue were small in proportion to the total ion uptake.

From the experiments it appeared:

a. that the ion uptake in the range investigated was directly proportional to the water uptake, independent of the way in which the water uptake was varied;

b. that the measure in which the transpiration stream was diluted with respect to the medium concentration was equal for all concentrations investigated.

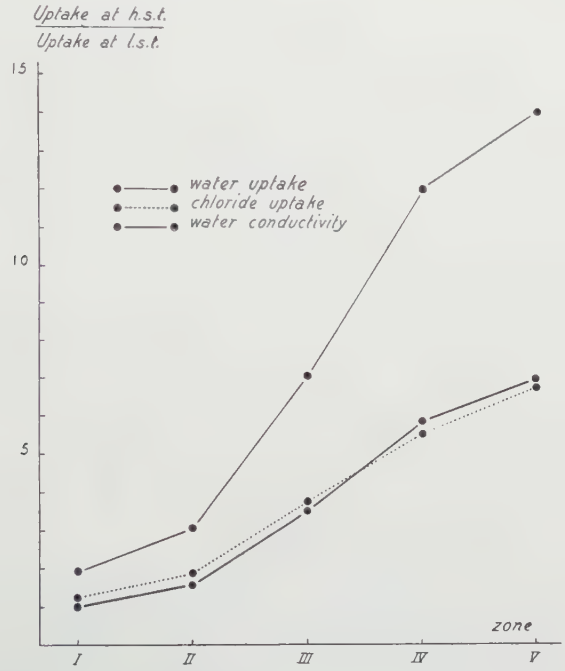

Fig. 3. Comparison of the ratio (mean value from about 40 experiments): $\frac{\text{uptake at high suction tension}}{\text{uptake at low suction tension}}$ for water and chloride by the various root zones with the ratio: $\frac{\text{waterconductivity at h.s.t.}}{\text{waterconductivity at l.s.t.}}$ Experimental conditions as in fig. 1 and 2 (first two periods). The ratio has been calculated by dividing the uptake values at high and low suction tension.

From the above HYLMÖ concluded that the influence of the water uptake on the ion uptake was direct and according as more water was taken up a proportional extra quantity of ions was carried along passively.

HYLMÖ's (1953) and BROUWER's (1954) experiments therefore correspond in so far that they both show a distinct correlation between transpiration and ion uptake. As was stated above the interpretation varied. This led HYLMÖ (1955) to examine my experimental results

again. According to Hylmö it would appear from this that it was also possible just as with *Pisum* to distinguish between the active and the passive components of the ion uptake with the aid of the data obtained for *Vicia faba*. The way in which this examination has been made will be critically discussed below. It will appear that the data examined by him yield no proof for his hypothesis concerning the mechanism of the ion uptake in *Vicia faba*. Some new results will be added.

II. Comments on Hylmö's publication

To discuss the most important items in Hylmö's publication (1955) it may be useful to follow it closely.

In figure 4 (Hylmö, 1955, fig. 1) the correlation between the water uptake and the chloride uptake inhibited or not has been plotted. Hylmö points out that on applying 2–4 dinitrophenol or on stopping

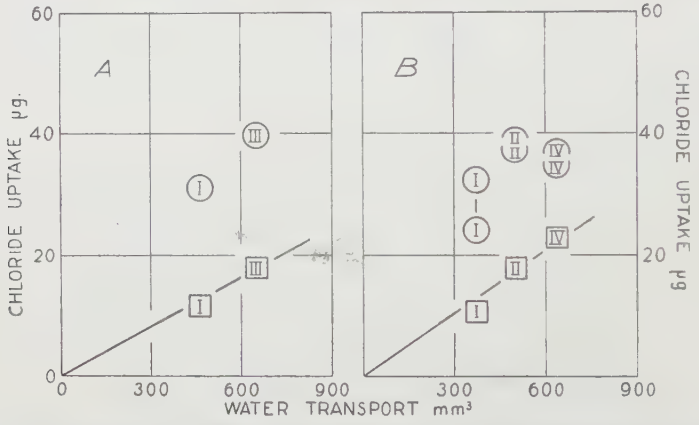

Fig. 4. The influence of 10^{-5} M 2,4-dinitrophenol (A) and of oxygen deficiency (B) on the water and chloride uptake of the different root zones at high suction tension in the vessels. Uptake per 3 cm of root length during 24 hours from a medium of 5 mM calcium chloride. Roman numerals designate the root zones counted from the root tip. A. Circles denote without DNP, squares with DNP. Data from Brouwer 1954 table IX A. B. Circles denote aerated roots, squares without aeration. Data from Brouwer 1954 table X. (Hylmö 1955 fig. 1, page 437).

aeration the cloride uptake is directly proportional to the water uptake, as is to be expected when the active component of this uptake is eliminated. Apparently he assumes that the active uptake is completely inhibited. No evidence however is present for this. Moreover it appears from the data of the non-inhibited uptake that it is also possible to connect this points by a straight line passing through the origin. This would mean that the whole uptake is passive, though sensitive to inhibitors. Here we meet an other objection to this figure, viz. a direct comparison of water and salt uptake of the various root zones, without its first being shown that the correlation between water uptake and salt uptake is equal for all zones.

Passing on to figure 5 we see that here too there are various objec-

tions to Hylmö's re-examination. The main objection is that in this figure the data from table I have been compared with the corresponding data from table II. These values have been derived from experiments with different plants and the values for the chloride uptake of the different roots vary greatly. This appears among other

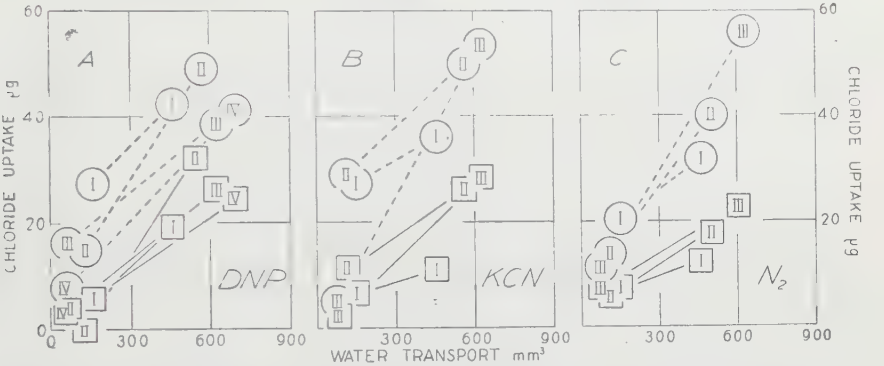

Fig. 5. The influence of 10^{-5} M 2.4-dinitrophenol (A), of 5.10^{-6} potassium cyanide (B), and of nitrogen bubbling through the medium (C) on the chloride uptake of the different root zones at low and at high water uptake. Uptake per 3 cm of root length during 24 hours from a medium of 5 mM calcium chloride. Roman numerals designate the root zones counted from the tip. Circles denote without inhibitors, squares with inhibitors. Data from Brouwer 1954 table VI. (Hylmö 1955 fig. 2, page 438).

things from the chloride uptake of the various zones without the use of inhibitors as have been given in the first columns of the different sub divisions of tables I and II, of which the most striking differences

TABLE I

Inhibition of the chloride uptake by various inhibitors at low water uptake. Chloride uptake of the different root zones with and without inhibitor

Zone	DNP 10 M			KNC 5.10 M			Nitrogen		
	—	+	% inh	—	+	% inh	—	+	% inh
IV	7	4	43				10	6	40
III	*16*	4	75	*5*	2	60	*11*	7	36
II	*15*	0	100	*29*	11	63	*13*	6	52
I	27	6	78	27	6	78	20	7	66

(Brouwer, 1954 table VI A)

TABLE II

Inhibition of the chloride uptake by various inhibitors at high water uptake. Uptake of the different zones with and without inhibitor

Zone	DNP 10 M			KCN 5.10 M			Nitrogen		
	—	+	% inh		+	% inh	—	+	% inh
IV	41	25	40	49	30	40			
III	*39*	26	35	*53*	28	47	*57*	23	60
II	49	32	37	59	26	48	40	18	55
I	42	19	55	36	11	70	31	12	61

(Brouwer, 1954 table VI B)

have been italized. So we see that a great difference in the chloride uptake of the various zones occurs in different experiments. Comparing these experiments with different roots seems therefore not permitted. Besides Hylmö took values for the water uptake which cannot be derived from the experiments from which the figures for the chloride uptake have been derived.

Another objection is just as in the case of the data of figure 4 that Hylmö assumes that the inhibitor concentrations used have just fully inhibited the active component of the ion uptake. He has no other indications for this than a number of data from literature by which this was assumed-though for other material. Side by side with it cases may be mentioned where much higher concentrations did not entirely inhibit the exudation (VAN ANDEL 1953). So Hylmö's figures though clear at first sight are no proof of his argument. As regards Hylmö's criticism concerning the use of sucrose as an osmotic substance to inhibit the water uptake, it is right that sucrose may have a twofold action i.e. beside the osmotic action also an influence on metabolism. I understood this objection as such and therefore used mannitol and magnesium sulphate as osmotica beside sucrose. Mannitol gave, probably owing to impurities in the preparation used by me at that time, no clear results, but magnesium sulphate did (BROUWER 1953*b*, p. 648). It is to be regretted that Hylmö neglected this last fact, just as LONG's observation (1943), who found for tomatoes that by sodium chloride just as by sucrose water uptake could be inhibited for 80 % without influencing the nitrate uptake.

At the conclusion of his paper Hylmö himself occupied with the changes in conductivity for water occurring in the root tissue. The correlation between water uptake and suction tension as it appears from my experiments, is an indication for him that we have to deal here with a phenomenon as was described by ERBE (1933) and PISA (1933) for ultra filters. This would mean that at low suction tensions only part of the microcapillaries in wall and plasm and that the larger ones join in the water transport. The greater the suction the more microcapillaries will join, till finally at a certain suction all microcapillaries are run through. From this moment there is a direct relation between suction tension and water uptake. This means, however, as well that at a low suction tension the water uptake per atmosphere is smaller than at a higher suction tension. Hylmö objects to using the term change in conductivity for this phenomenon. In literature, however, this conception "change in conductivity" has been repeatedly used (RENNER, 1929; BRIEGER, 1928; KOEHNLEIN, 1930; BREWIG, 1937; 1939; and KRAMER, 1949). ARISZ (1956) has pointed out that in physiological literature terms are often being used without there being question of a complete knowledge of the phenomena they refer to. So here too the term conductivity has been used in a purely descriptive way. Therefore in connection with the fact that for the water supply of the plant a higher suction tension per atmosphere suction is more efficient than a low suction tension, we can speak of an enhanced conductivity for water. The question

remains what this enhanced conductivity is based on. It may be due to the joining of a greater number of microcapillaries. The data on the influence of the turgor on the water conductivity, however, as described in a previous publication (Brouwer, 1954*a*), tell against this.

III. The influx coefficient of the various zones

The notion influx coefficient, i.e. "the concentration of the true transpiration stream from the medium to the root in relation to the concentration of the medium", is ascribed great value to by Hylmö (1955, p. 443) when trying to prove the passive nature of the ion uptake. From my experiments with different plant material, however, my experience is that this quantity is most variable, dependent as it is on the salt status of the material. In Pisum plants poor in chloride I found an influx coefficient of 0.52, where as the same material when cultivated on a calcium chloride solution for some time after, showed an influx coefficient of 0.03. For rye plants poor in chloride and rich in phosphorus the influx coefficient for chloride amounted to 1.64 and at the same time for phosphate to 0.2. Conversely these values amounted to 0.16 and 1.72 resp. for rye plants rich in chloride and poor in phosphate. From Hylmö's data (1953 fig. 7) there also appears a certain variability. On the whole the influx coefficient decreases with a rising medium concentration and an increasing saturation of the plant with a certain ion.

When examining my experiments on the uptake by the various zones of the root of *Vicia faba* Hylmö arrives at the conclusion that the influx coefficient of the various zones is the same. This conclusion is based on the data given in table III. This table, however, has

TABLE III

The influx coefficients of the different zones as calculated from various experiments

Figure no.	Zone I	Zone II	Zone III	Zone IV
2 A	0.14	0.21	0.16	0.14
2 B	0.09	0.14	0.24	—
2 C	0.10	0.18	0.23	—
3	—	0.12	0.08	0.12
4	0.05	0.08	0.09	0.09

(From Hylmö 1955 p. 443, after tables and figures of Brouwer 1954)

been composed by Hylmö among other things at the hand of the uptake values of figures 4 and 5 discussed above. So the criticism given here is also applicable to the use of this table. Moreover, as already observed by Hylmö, the number of observations is too low to make conclusions possible. A more reliable and at the same time simpler method to get an insight into the influx coefficient of the various zones, is to start from figures 1 and 6. Brouwer, 1954*b* figs. 6 A and 6 B), as a result of which we get figures 7 A and 7 B. Here data on salt and water uptake determined at the same root have been used. The result is that a difference in values of the influx coefficient can be observed right enough (difference in slope of the

lines). Yet even now a few objections have to be made. The most important being that the regression lines indicating the correlation between water uptake and cloride uptake, just as is the case in Hylmö's figures, pass through two points only.

In figure 8 the average chloride uptake of all (26) experiments with 5 root zones with low and with high transpiration is plotted

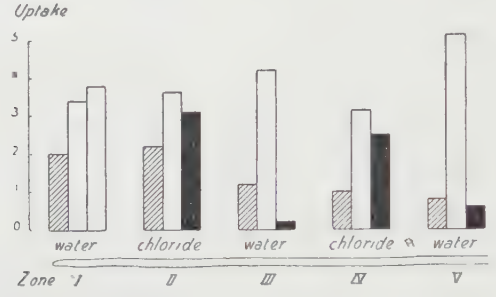

Fig. 6. Influence of an osmotic counter suction with sucrose in the nutrient solution on the water and chloride uptake at high suction tension in the xylem vessels. Chloride and water uptake of the various zones of the root at low suction tension (shaded blocks), at high suction tension without sucrose (white blocks) and at high suction tension with an osmotic counter suction (black blocks) in the medium. The sucrose solution strongly decreases the water uptake without a reduction of the chloride uptake. Scale unity 14 γ Cl or 200 mm^3 of water per zone per 24 hours.

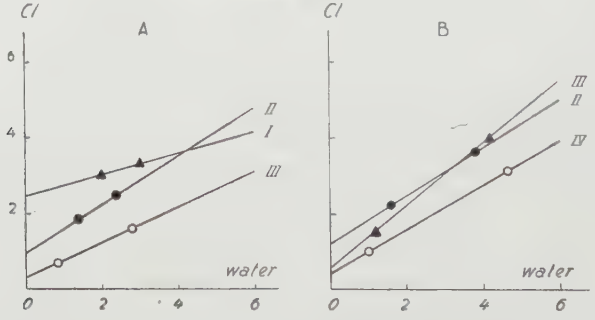

Fig. 7 A. The relationship between water and chloride uptake of the various root zones at low and at high suction tension in the xylem vessels. Data from figure 2 first two periods. Chloride uptake by zone II calculated by interpolation between zone I and III. Water uptake by zone I extrapolated, water uptake by zone II and IV interpolated.

Fig. 7 B. As in figure 7 A. Data from figure 6. Chloride uptake by zone III and water uptake by zone II and IV attained by interpolation. Scale unity vide Figure 1.

against the average values of the water uptake of all (84) experiments with 5 root zones likewise with low and with high transpiration. Because of these greater numbers it may be expected that the result is statistically more reliable than in the four experiments on chloride uptake collected by Hylmö (table III). Also in figure 8 it strikes the

eye that the regression lines for zones I and II are less steep than those of the more basal zones. The influx coefficients from top to base amount to 0.054; 0.074; 0.13; 0.13 and 0.13 resp. So we may conclude from figures 7 and 8 that the correlation between water uptake and chloride uptake for the various zones does not show the same picture. So a uniform filtration of the medium solution over

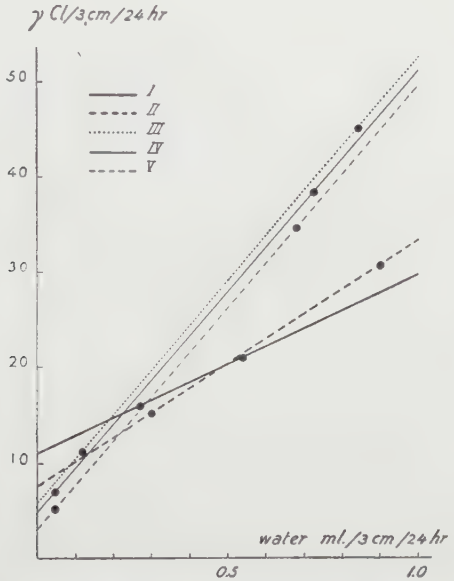

Fig. 8. Mean values of the water uptake by the various root zones versus the mean values of the chloride uptake.

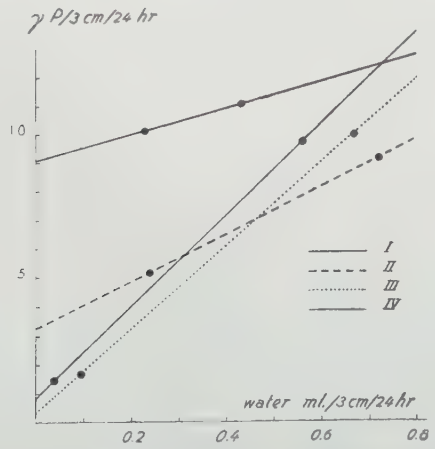

Fig. 9. Mean values of the water uptake by the different root zones versus the phosphate uptake.

the entire root length is out of the question. Figure 9 gives the result of an identical revision of the phosphate experiments. The computed values of the influx coefficient amounted to 0.31; 0.50; 0.91 and 1.0 from top to base. These are much higher than those found for chloride. This is probably due to the fact that the plants had been cultivated on tap water which in contrast to Hylmö's supposition (Hylmö 1955, p. 442) did contain chloride and no phosphate. From these phosphate experiments it is again clear that the influx coefficients of the various zones need not be identical.

On the ground of the above we can determine that Hylmö's reexamination of data previously published by me cannot yield any proof for the occurrence in *Vicia faba* of a passive component of the ion uptake linked directly to the waterstream.

Without further preface it is of course a fact that there is a strong correlation between water uptake and ion uptake. The direct causal relation as Hylmö thought to demonstrate from my data has not been proved and continues to be open for discussion. In the following chapters I shall describe fresh experiments (all carried out with *Vicia faba*) and bring up for discussion the interpretation that may be given to them.

IV. The relation between water uptake and chloride uptake

In the previous chapter it appeared that a regression line which is fixed by only two points insufficiently represents the entire correlation between water uptake and chloride uptake. In the experiments described below it has been tried by taking several periods and by varying the water uptake in different ways to meet this objection. The design of these experiments may best be explained by a detailed example as given in figures 10, 11 and 12. Figure 10 gives the chloride uptake of the zones I, II, IV and V from a 5 mM calcium chloride solution for five successive periods (A, B, C, D, and E), the amount of water taken up by zone III in the same periods and from the same solution being measured. The nutrient solution was constantly being aerated and the shoot constantly exposed to light. The suction tension in the plant and as a result the water uptake by the tested root was varied by bringing into the main vessel tapwater (A), 2 atm. sucrose (B), tapwater (C), 3 atm. sucrose (D) and tapwater (E) resp. In addition the growth in length of the apical zone has been plotted as a dotted line in figure 10 A at the foot. As for the uptake we see that the chloride uptake of the zones II, IV and V is correlated in the main with the water uptake by zone III. The chloride uptake by zone I is more related with the growth in length of that zone. It strikes the eye that the growth in length of the top is strongest in the periods in which there is tapwater in the main vessel i.e. at a slight suction in the plant and smallest at a high suction tension in the plant (sucrose in the main vessel). In the right half of figure 10 (B) the chloride uptake of each zone has been plotted for each individual period. With the exception of zone I, in which growth in length occurs, the various zones give very regular pictures. It seems

therefore permitted to compute the chloride uptake by zone III by interpolation of the uptake values of zones II and IV. This chloride uptake by zone III obtained by interpolation has been plotted in figure 11 against the measured water uptake of this zone. Now it appears from figure 11, that the correlation between water uptake and chloride uptake is not directly proportional. The point of

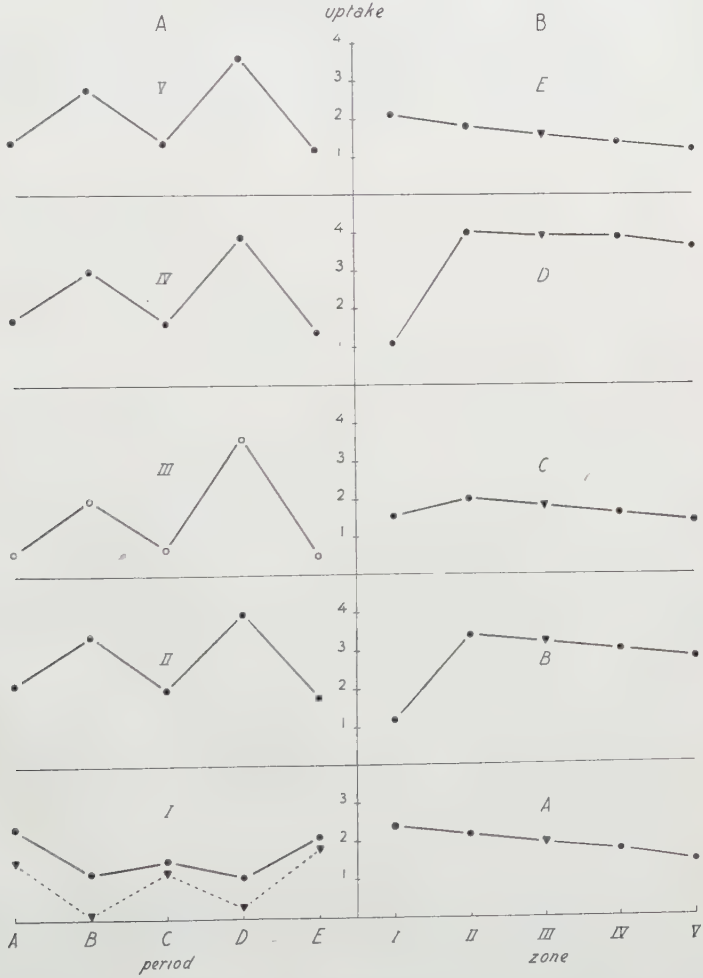

Fig. 10 A. The chloride or water uptake of different zones of the tested root in successive periods (A–E) at various osmotic values of the solution in the main vessel. A, C and E tap water in the main vessel, B 2 atm sucrose and D 3 atm sucrose. ○——○ water uptake; ●——● chloride uptake; ▼——▼ longitudinal growth of the tip zone. Scale unity 14 γ Cl, 200 mm^3 water or 5 mm growth per zone per 24 hour.

Fig. 10 B. The chloride or water uptake as found in fig. 10 A during the different periods. Interpolation of the chloride uptake by zone II and by zone IV gives the chloride uptake by zone III (▼).

intersection of the two regression lines drawn with the y-axis shows great differences in the accumulation in the root to be expected from these lines, viz. 12.0 and 21.5 γ chloride. In figure 12 the chloride uptake have been plotted against the suction tension measured in the xylem vessels (method see BROUWER, 1953*a*, *b*). From this it appears that the water uptake follows a course as is to be expected with the occurrence of changes in conductivity, i.e. slight suction tensions are relatively less effective (points under the straight line) than the higher ones. At higher suction tensions the chloride uptake reaches a kind of saturation value (dotted line). The line drawn for the chloride uptake shows a great correspondence with the correlation

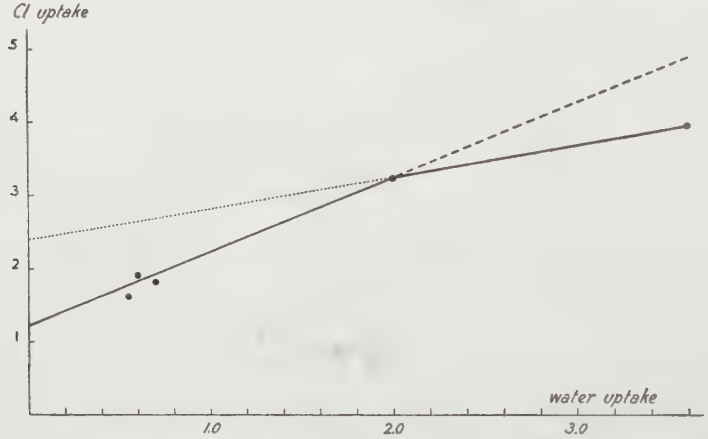

Fig. 11. The relation between the water uptake by zone III from figure 10 A and the chloride uptake by this zone as calculated from figure 10 B.

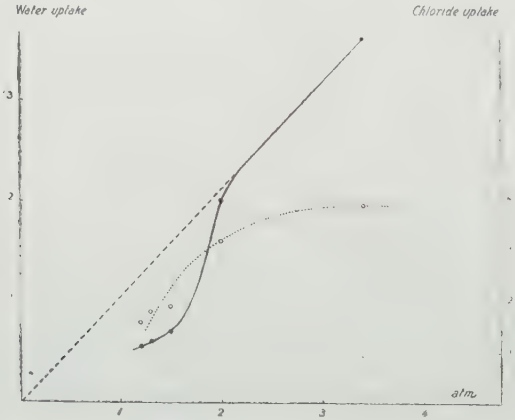

Fig. 12. The water uptake (full line) and the chloride uptake (pointed line) of zone III (vide figures 10 and 11) versus the suction tension in the xylem vessels. The dotted line represents the water uptake as would be expected without changes in water conductivity.

between suction tension and water conductivity (see BROUWER 1954*b*, fig. 2). How great this correspondence is for the case in question, may appear from figure 13 which gives the correlation between suction tension and chloride uptake as a dotted line, the correlation between suction tension and water conductivity as a solid line. A certain parallelism is unmistakable. It seems that with the higher values of the suction tension the chloride uptake relatively increases a little more than the water conductivity. For the significance of this phenomenon I refer to the discussion. Lastly figure 14 may serve as an example to make clear that even three points are not yet sufficient to fix the entire correlation between water uptake and salt uptake. It looks like it that this relation should be rendered by a curved line.

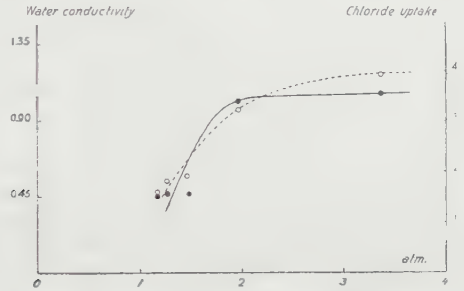

Fig. 13. Chloride uptake (dotted line) and water conductivity (full line) versus suction tension (data from figures 10, 11 and 12). Scale unity 200 mm³ water, 14 γ Cl and 1 mm³/1 cm/1 atm/20 minutes.

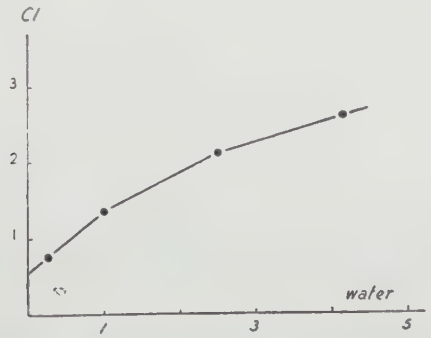

Fig. 14. The chloride uptake by the third zone of another experiment at various levels of the water uptake. Scale unity vide figure 1.

V. THE RELATION BETWEEN WATER UPTAKE AND CHLORIDE UPTAKE WHEN 2.4-DINITROPHENOL (DNP) IS USED

In a previous publication (BROUWER 1954*b*) it was shown that 10^{-5} DNP administered for one period inhibited the chloride uptake to about 50 % (varying between 35 and 100 %) without influencing the water uptake. On prolonged application a further inhibition follows some influence on the water uptake becoming visible as well.

This has been rendered in figures 15 A and 15 B. Here the water uptake (open dots) and the chloride uptake (black dots) of the various zones in successive periods has been plotted. During period A the uptake was determined from a 5 mM calcium chloride solution to which during the following periods DNP was added to a concentration of 10^{-5} M. The data have been obtained from two parallel experiments A and B, in which in the experiment of fig. A the water uptake was

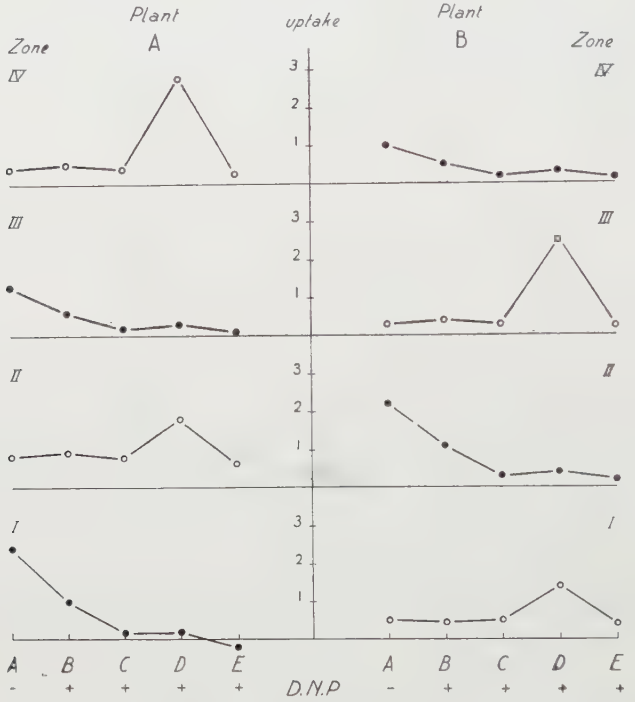

Fig. 15 A and B. The chloride and water uptake by the various root zones of the tested root at low and high suction tension during successive periods from a 5 mM calcium chloride solution (period A) and from this solution with addition of 10^{-5} DNP (periods B–E). Open circles denote water uptake, black dots chloride uptake. Scale unity vide figure 1.

determined of zones II and IV and the chloride uptake of zones I and III, whereas in fig. 15 B just the reverse took place. During the periods A, B, C and E the suction tension was kept low, during period D it was increased. This latter is clearly shown by a strongly increased water uptake, whereas the increase in chloride uptake in this period is slight. Under the influence of DNP the correlation between chloride uptake and water uptake has practically disappeared here. The correlation between chloride uptake and water uptake with and without DNP being administered, has been represented in fig. 16. As for its arrangement this experiment was identical with the experiment plotted in the figures 10 up to and including 13. For five successive

periods the chloride resp. water uptake of the various zones was determined. The chloride uptake of zone III (obtained by interpolation) has been plotted against the measured water uptake of that zone. During periods A and B the uptake was determined from a 5 mM calcium chloride solution only whereas in periods C, D and E DNP had been added. During periods A, C and E there was tapwater in the main vessel, during periods B and D 0.1 M sucrose. From figure 16 it appears that the line through A and B shows the

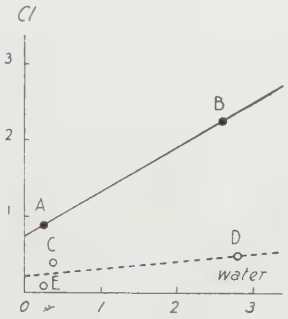

Fig. 16. The relationship between water uptake and chloride uptake with (open circles) and without (black dots) 2,4 dinitrophenol addition to the 5 mM calcium chloride solution. Scale unity vide figure 1.

normal relation between water uptake and chloride uptake. The chloride uptake during period C has been inhibited for about 50 % in respect of that of period A. Indeed during period E, under identical circumstances the chloride uptake has still further decreased. Comparing the average of the uptake in periods C and E with the uptake in the intermediate interim period D seems reasonable. This then shows the correlation between chloride uptake and water uptake in the presence of DNP, as it may be found in figure 16 as a dotted line. There is a great difference in slope between the lines A–B and C/E–D. With an equally strong rise of the water uptake the chloride uptake without inhibitor increases much more than the one with inhibitor. The influx coefficients computed from these lines amount to 11.6 % (A–B) and 1.7 % (C/E–D) of the medium concentration resp.

VI. The correlation between water uptake and chloride uptake when different osmotica are used

In a previous publication it was shown that when the water uptake was inhibited by sucrose solutions the chloride uptake hardly decreased. It was even proved possible to decrease the water uptake and at the same time increase the chloride uptake in some circumstances by administering sucrose. As an explanation of this phenomenon it was thought at the time that a change in the conductivity for salts, under influence of the osmotic action of the sucrose increases the salt uptake. This was in entire agreement with the observation that sucrose increases the conductivity for water (Rosene 1941,

Brewig 1939, Brouwer 1954*a*) and as a result of an unaltered mechanic suction in the xylem vessels the water uptake as well. In the undermentioned experiments it has been tried to extend the earlier data in that sense that of the same root data were obtained about water uptake and chloride uptake with and without osmotic counter suction in the root medium, both combined with low and high suction tension in the xylem vessels. Besides sucrose there were used as osmotica: lactose, mannitol and magnesium sulphate. This variation was chosen to enable us to separate a possible influence on the metabolism from a purely osmotic influence. Mannitol and magnesium sulphate had also been used for this purpose in the investigation published in 1954, but at the time the results with mannitol were rather variable, so that sometimes even a loss of chloride was found, sometimes, however, the same picture was obtained as in the case of sucrose. A protracted treatment with the mannitol used by me at the time showed a colouring black of the roots, a phenomenon that was never observed when sucrose was used. These experiments have now been repeated and extended. The results of some of these experiments have been plotted in figures 17 and 18. Just as in the previous chapters the interpolated value of the chloride uptake has been plotted in these figures against the water uptake measured. Points 1 and 2 have been obtained at a low

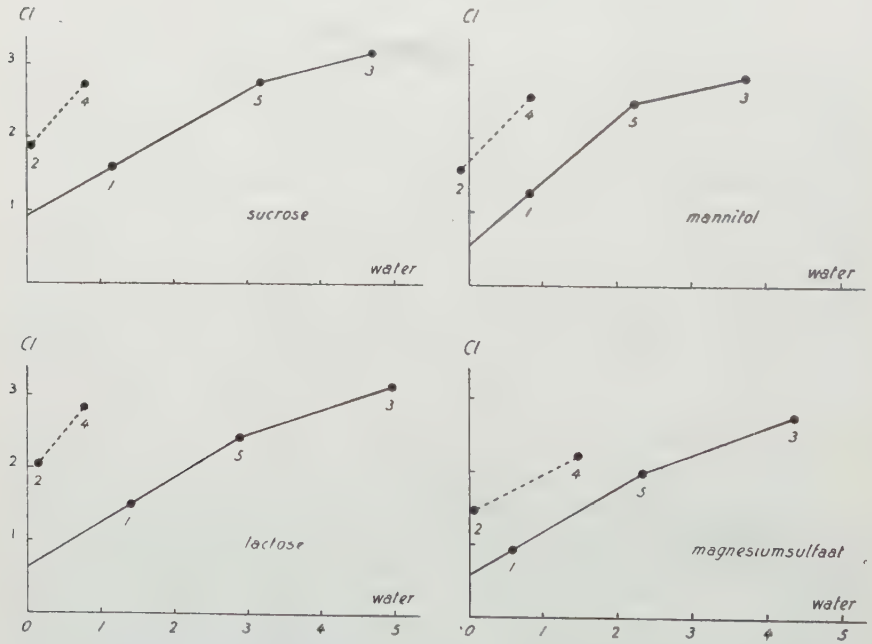

Fig. 17. Water and chloride uptake by zone III at various levels of the suction tension with or without an osmotic counter suction in the medium. The water uptake has been measured directly, the chloride uptake has been attained by interpolation. Scale unity vide figure 1.

mechanic suction in the xylem vessels, 4 and 5 at a higher one and 3 at the highest. During periods 1, 3 and 5 the uptake was determined from a 5 mM calcium chloride solution on addition of the osmoticum to a concentration corresponding with 2.5 atm. On comparison of the points 5 and 3 with 2 and 4 resp. (fig. 17) we see that owing to the osmotic counter suction in the medium the water uptake has been greatly diminished, much more than the chloride uptake. This holds good both for sucrose and for lactose, mannitol and magnesium sulphate. In all these cases therefore the chloride uptake is much higher than might be expected on the ground of the regression lines through the points 1, 5 and 3 with the water uptake of periods 2 and 4. So the first result from these experiments is that by applying osmotically active substances of a various nature the chloride uptake is higher than fits the inhibited water uptake. Further the points 2 and 4 have been connected by a line. These lines give the correlation

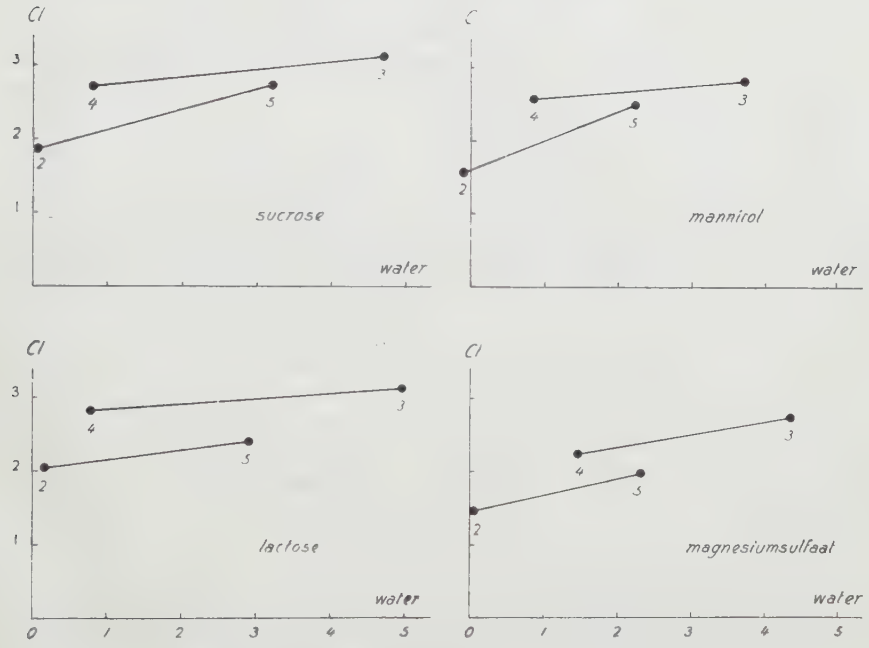

Fig. 18. The same data as given in figure 17.

between water uptake and chloride uptake at an osmotic counter suction in the medium. Here we also see that according as more water is taken up there is a greater ion uptake. The slope of these lines is steeper for sugars than that of the lines through points 1, 5 and 3. This is not the case for magnesium sulphate. We have now compared the relation between water uptake and chloride uptake with and without counter suction in the medium. In figure 18, four of the five points of figure 17 have been laid of again. Now the points 4 and 3 and 2 and 5 resp. have been joined. The train of thought

underlying this is that these points in twos correspond with about equal suction tensions in the root tissue. For the points 2 and 5 it will amount to about 2.5 atm. for the points 4 and 3 rather more than 3 atm. We now see that under these circumstances there also exists a correlation, be it less pronounced, between water uptake and chloride uptake.

VII. The fate of the ions in the plant

The experiments described above in which the ion uptake of individual root zones was compared with the water uptake of those zones, do not give a direct insight into the distribution of the absorbed ions in the plant. In order to get an impression of this, some experiments will be described here, in which this is the case. These experiments were made with rubidium chloride in which the rubidium was labelled. For the rest the arrangement of the experiments was such that the whole root system was put in the nutrient solution. To this nutrient solution sucrose was added or not, the influence of aeration being examined with presence and absence of sucrose. In a previous publication it was already indicated that experiments in which the whole root system was put in the nutrient solution also showed a distinct correlation between water and chloride uptake. At the same time the influence of sucrose and magnesium sulphate on the chloride uptake was examined. The results of such an experiment have been plotted in figure 19. The regression line has been determined by measuring the chloride uptake (analysis of the medium) and the water uptake of plants 1 and 2 in the case of low (1.2) and

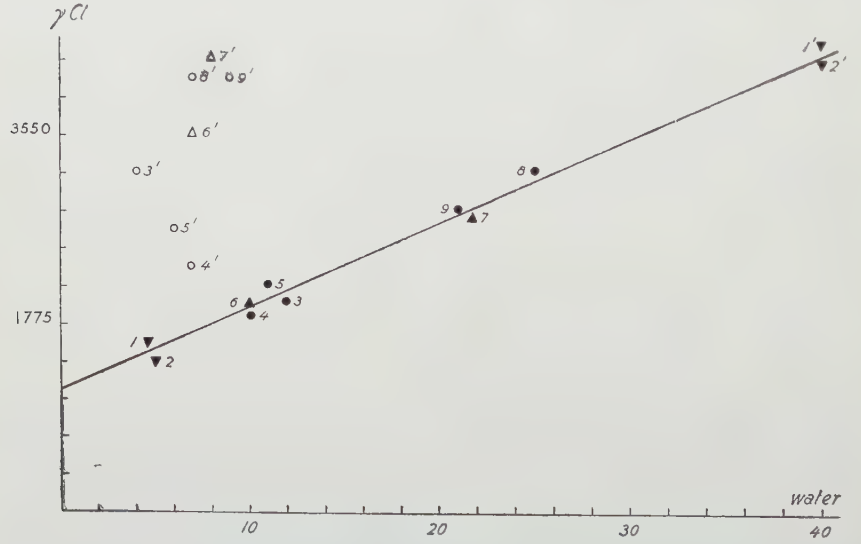

Fig. 19. Water and chloride uptake by the whole root system of *Vicia faba* plants in two successive periods. Each numeral corresponds to one plant. Nutrient solution: plant 1–9 and 1′–2′ 5 mM $CaCl_2$, 3′, 4′, and 5′ 5 mM $CaCl_2$ + 2 atm sucrose, 8′ and 9′ 5 mM $CaCl_2$ + 2.7 atm sucrose, 6′ and 7′ 5 mM $CaCl_2$ + 2.7 atm $MgSO^4$

in the case of moderate (1′, 2′) transpiration. Of plants 3, 4, 5 the uptake from a 5 mM calcium chloride solution has first been determined and in the second period from the same solution with addition of 2.0 atm. sucrose (3′, 4′, 5′). It appeared that this treatment reduced the water uptake and increased the chloride uptake (points over the line). With plants 8 and 9 the same method was followed, but now I used in the second period 2.7 atm. sucrose (8′, 9′) instead of 2.0. We see a relatively more strongly reduced water uptake and a still greater chloride uptake. With plants 6 and 7 magnesium sulphate (2.7 atm.) was added to the calcium chloride solution instead of sucrose in the second period. Here too we see a result corresponding with that obtained with sucrose, a reduced water uptake and a relatively increased chloride uptake (6′, 7′). It always strikes us that on our using an osmotic counter suction in the medium the chloride uptake is higher than corresponds with the water uptake concerned without counter suction. So the correlation between water uptake and salt uptake is modified by this treatment.

Before passing on to the results of the rubidium experiments we should point out that results obtained with rubidium do not simply hold good for chloride as well. The plants used in the experiments were cultivated on Rb free but Cl containing nutrient solution (tap water). Especially the fixing in the root may be for Rb different from what it is for Cl (cf. Hylmö's results with Ca and Cl in *Pisum*). Further in all previous experiments uptake values of one plant or of one zone have always been compared (with each other) in successive periods. In order to follow the fate of the ions in the plant, the plants must be sacrificed. In the results therefore the variability of the plants

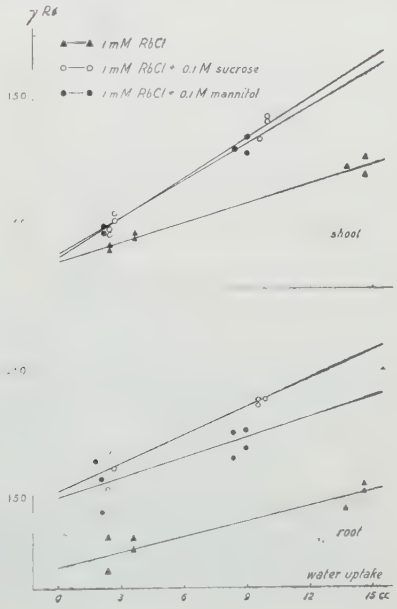

Fig. 20. Water uptake and rubidium uptake (root and shoot separated) by intact Vicia faba plants. Each dot represents the mean uptake by 4 selected plants. Nutrient solution:

▲——▲ 1 mM RbCl,
○——○ 1 mM RbCl + 0.1 M sucrose
●——● 1 mM RbCl + 0.1 Mm annitol

is included. In order to limit these differences as much as possible averages of 4 plants strictly selected for the experiment were used for each observation. As nutrient solution 1 mM Rb Cl was used; the plants were constantly exposed to the light. The water uptake was determined by weighing. The duration of the experiment was 20 hours, the root systems being washed after that on distilled water for $1\frac{1}{2}$ hours to remove Rb that could be washed out. Figure 20 gives the results of an experiment in which the Rb uptake into the plant and the distribution over root and shoot was traced at low transpiration (under a glass bell) and at evaporation free in the air. The nutrient solution was 1 mM Rb Cl without addition (triangles) with addition of 2.5 atm. mannitol (black dots) or with addition of 2 5 atm sucrose (open dots). Both sucrose and mannitol give an increased accumulation in the root together with an accelerated transport to the shoot. The correlation between rubidium uptake and water uptake is more pronounced on addition of sucrose and mannitol than without these substances. Figure 21 gives the result of an experiment in which

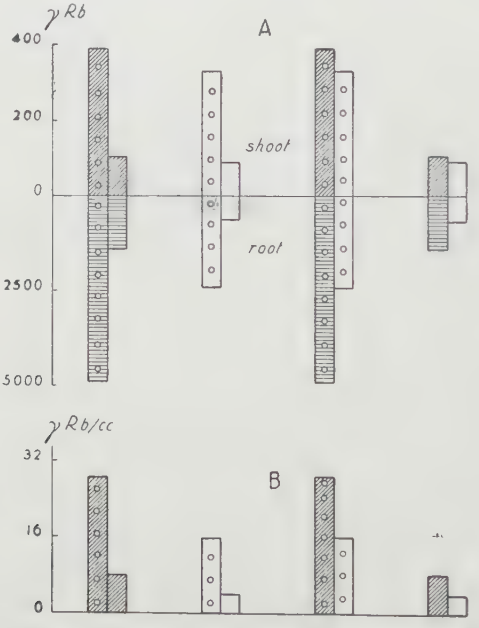

Fig. 21 A. The rubidium contents (roots and shoots) of *Vicia faba* plants after an uptake period of 20 hours on 1 mM rubidium chloride at moderate transpiration with (bubbles) and without aeration, both with (shaded) or without (white) 0.1 M sucrose.

Fig. 21 B. The rubidium transport to the shoot per ml water transpired (data from figure 21 A divided by the amount of water taken up).

under constant circumstances for the shoot (light, free to the air), the Rb distribution was examined after an uptake period on Rb Cl without addition (white blocks) and one with addition of 2.5 atm. sucrose (shaded blocks) both with and without aeration.

Figure 21 A gives the distribution of Rb over root and shoot under the various circumstances. Sucrose (left half of the figure) gives a greatly increased accumulation in the root and a somewhat greater transport to the shoot. The influence of the aeration, both on the accumulation in the root and on the transport to the shoot is very clear (right half of the figure). In figure 21 A the water uptake has been left out of consideration. The water uptake was almost equal with or without aeration, sucrose causing a 35 % reduction in the water uptake. In figure 21 B the concentration of the transpiration stream has been given, i.e. the amount of rubidium finding its way into the shoot per ml water which has been evaporated. This figure 21 B is most interesting for our problem. If we first examine the sucrose influence (left half of the figure), it appears that on addition of sucrose both with and without aeration the concentration of the transpiration stream is nearly doubled. Sucrose therefore has a very pronounced influence on the concentration of the transpiration stream, a fact already apparent from the slope of the regression lines of figure 20. Even stronger than by the sucrose influence we are here struck by the influence of aeration (right half of figure 21 B). Both on application of sucrose and without it the concentration of the transpiration stream has been reduced to less than a third by our leaving out aeration. It strikes us in figure 21 A that an increase in Rb uptake going together with aeration or administration of sucrose, has benefited root and shoot in about the same degree. It may be concluded therefore that the concentration of the transpiration stream may be considerably increased by sucrose and mannitol and may be considerably reduced by putting a stop to aeration.

VIII. Discussion

In literature we find no uniformity of opinion on the mechanism of ion uptake by the root. Treating the various theories is outside the purpose of this investigation. In a previous article (Brouwer 1954*b*), just as in Hylmö's (1953, 1955), Petritschek's (1954) and van den Honert's (1955) papers a special study was made of the transpiration on the ion uptake. Arisz (1945, 1956), Burström (1954, 1955) and Huber (1953) have also paid special attention to this phenomenon in reviewing articles. Except van den Honert the above mentioned investigators found a distinct correlation between water uptake and salt uptake. As for the question why in some cases this correlation is lacking, van den Honert submits different possibilities for consideration, while the author on the ground of various experiments (Brouwer, 1953*b*), especially looks for an explanation via the state of the ions in the plant. This point will not be considered here any further. We start, however, from the supposition that the ion uptake can show a pronounced correlation with the water uptake.

Now the interesting point is whether the experiments described above can give some further indications on the nature of this correlation. For the question is whether this correlation is based on the occurrence of a passive component in the ion uptake, or if a

relation is found between the active ion uptake as well. According to Hylmö the water uptake promotes the accumulation in the root only if it contains but little of the ions concerned. If there is a promotion of accumulation this should be attributed to a penetrating of ions to root parts which would not be reached without a strong water uptake. Beside it the transport of ions to the shoot is nearly completely passive. A removal of ions from the root accelerated by transpiration, ions which were actively fixed there beforehand or were actively taken up, is rejected by him. Petritschek on the other hand finds facts which indicate that ions which have been fixed in the root at night, are given off to the transpiration stream in the day time and next transported to the shoot. As explained in the introduction, I thought to be allowed to conclude from my experiments that the correlation between water uptake and ion uptake was based on a rise in active uptake. Though Hylmö does not accept this view in its general tendency, he himself comes to the conclusion (Hylmö, 1955, p. 442) that in my experimental results there are indications which render an increase in bleeding (active uptake different from accumulation in the root) parallel to the increased transpiration possible. In his final conclusions however, Hylmö does not revert to this, so that his article as a whole arouses the impression that an increase of the active transport to the shoot at increasing transpiration should be entirely rejected in his opinion. On the other hand I thought I had to explain the whole phenomenon exactly in this way. Further it is also possible that both factors act a role while finally the possibility should be considered if all plants behave in the same way (Arisz, 1956).

The meaning of this new research was to gather further data on a possible separation between active and passive components in the ion uptake of *Vicia faba*. For this especially those experiments are important that render a determination of the ion transport to the shoot possible. In first instance they are the experiments taken with rubidium. In those the direct transport to the shoot was determined. Now it appears from figure 21 that by stopping the aeration of the root medium the concentration of the transpiration stream is reduced to $\pm$ 30 %. This indicates that at any rate some 70 % of the ions, owing to a mechanism controlled or started by metabolism lands into the transpiration stream. Probably this percentage is still higher, seeing through stopping the aeration the oxygen concentration does not become nihil. The latter as follows from the fact that under these circumstances accumulation can still be observed in the root tissue. So it looks as if an active component also acts an important part in the ion transport to the shoot. In the same direction points the influence of sucrose on the concentration of the transpiration stream. Figure 21 B gives direct data of this too. Applying sucrose doubles this concentration. Here too the most obvious explanation is that an active process is to a high degree responsible for the ion concentration in the xylem vessels. In the experiments plotted in figure 20, a comparison has been made between sucrose and mannitol, in which

the phenomenon occurs that these two substances have a similar be it not quite equivalent effect. We do not directly expect mannitol to influence metabolism. ARISZ and SOL (1956) however also find in *Vallisneria* a stimulating effect of mannitol on the chlorid accumulation. So we must consider an influence on metabolism possible.

The experiments discussed above refer to rubidium transport which as has been said could be determined directly. This was not the case in the chloride experiments. Yet in those we can also get an impression of the chloride transport to the shoot from the correlation between chloride uptake and water uptake. Figure 16 clearly shows that by the use of 2.4 dinitrophenol the correlation between water uptake and ion uptake gets much smaller. This points distinctly to a decrease in concentration of the transpiration stream by inhibitors. Here too the conclusion is obvious that a considerable part of the ions is landed into the transpiration stream by an active mechanism. In the same way figure 17 shows clearly that sucrose, lactose and mannitol increase the slope of the regression lines i.e. they raise the concentration of the transpiration stream.

In order to give an impression of the quantitative relations at issue here, the concentrations of the transpiration stream computed from the various experiments have been gathered in the subjoined table IV. In the centre column the concentration has been given found simply on using the aerated nutrient solution. The concentration of the transpiration stream oscillates in this case between 9.2 and 18.8 % of the medium concentration. If aeration is stopped or an inhibitor given, this concentration falls considerably viz. to 1.7–4.7 %

TABLE IV

Influence of inhibitors and sucrose on the concentration of the transpiration stream as percentages of the medium concentration

	Nutr. sol non aerated	Nutr. sol + DNP	Nutr. sol aerated	Nutr. sol + 0.1 sucr. aerated	Nutr. sol + 0.1 sucr. non aerated
Cl-uptake		1.7	11.6		
Cl-uptake			12.1	26.6	
Cl-uptake	4.1		14.7		
Rb-uptake			9.2	18.8	
Rb-uptake	4.7		18.8	33.8	9.4
Rb-uptake	3.5		14.7	25.3	6.9
Rb-uptake			10.1	19.5	

of the medium concentration. This means that this concentration with respect to the control has been decreased by 70–80 %, the ion transport with equal water uptake being inhibited to this percentage. Conversely it appears from the values in the fourth column that sucrose about doubles the concentration, while the fifth column shows that the inhibition in the presence of sucrose again amounts to 70 %. In my opinion these data clearly show that at least 85–70 % of the ions is landed in the transpiration stream by an active process.

Surveying these results, we have therefore settled that there exists

a very distinct correlation between water uptake and ion uptake, but at the same time that a passive carrying along of the ions in the transpiration stream must be very much limited. How can we then explain this correlation? In 1954 I ascertained that there exists a strong correlation between the increase in chloride uptake and the increase in water conductivity as it may appear from figure 3 and is corroborated by the results laid of in figures 12 and 13. As to the nature of this correlation we are in the dark. The hypothesis, however, was made that the increased ion uptake was due to an enhanced conductivity for salts, just as an enhanced conductivity for water increases the water uptake per atmosphere suction. Now we know of the water conductivity that on a rise in suction tension it initially increases rapidly to reach a maximum value at last. This maximum value is attained in the suction tension range of 2.5–3.0 atm. But before it had already been found that the apical zone on a rise in suction tension after an initial rise in conductivity showed a fall (fig. 22 A). At the time I attributed this to an injury to the apical zone when closed in. Afterward it appeared to be a reversible phenomenon which may also be found in zone II (fig. 22 B). The

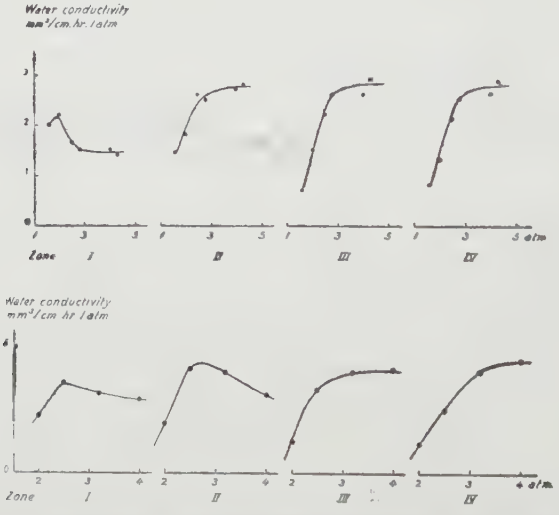

Fig. 22 A en B. Relationship between water conductivity of the various root zones and the suction tension in the xylem vessels. This relation has been plotted for each zone separately. All the values plotted are determined at a steady state, the water uptake and the suction tension being constant for several hours. (fig. 22 A from Brouwer 1954 fig. 2 and fig. 22 B from Arisz 1956 fig. 15)

relation between suction tension and water conductivity is therefore probably given by an optimum curve. I have never succeeded in increasing the suction tension in such a way, that the water-conductivity of the more basal zones also started decreasing. If the salt uptake is quite or for the greater part determined by the conductivity for salts and if the supposition is true that conductivity for

water and for salts are effected in the same sense and in the same measure by the suction tension in the tissue, it is to be expected that in the range of the high suction tensions the salt uptake does not increase any more at a rising suction tension whereas the water uptake does. This means that at a high suction tension no correlation is expected between salt uptake and water uptake. The chloride uptake as plotted in figures 11 and 12 is in agreement with this expectation. The salt uptake increases strongly in the suction tension range in which the water conductivity also strongly increases, but alters little in the higher suction tension sphere. This tallies with the result of the experiment laid of in figure 14. The correlation between water and salt uptake is strongest with lower values of the water uptake (low suction tension range) and decreases with greater water uptake (high suction tension sphere). Figure 18 also gives an indication in this direction. Here points are connected of which it may be supposed that the suction tension in the tissue is about equal. The highest suction tension we found at points 4 and 3. The slope of the line giving the relation between water uptake and chloride uptake under these circumstances, corresponds with an average influx coefficient of 2.27 % (sucrose 2.3 %; lactose 1.4 %, mannitol 1.75 % and Mg SO 3.7 %). The slope of the lines through the points 2 and 5 is averagely somewhat greater and corresponds with influx coefficients of averagely 4.7 % (sucrose 4.6 %; lactose 2.6 %; mannitol 7.8 % and Mg SO 3.7 %). If the salt uptake should be completely determined by factors which also determine the water conductivity we should not expect here a rise with increasing water uptake and the lines would have to run horizontally. This is not quite the case, there is only question of a strong reduction of the influx coefficient. Comparison of the results as they have been plotted in figures 17 and 18, gives the values of the influx coefficients given in table V for the various lines. From top to base the suction tension range lies higher and entirely in correspondence with the influx coefficient lower.

TABLE V

Influence of the suction tension on the influx coefficient (figures 17 and 18)

Regression line	Influx coefficients			
	sucrose	lactose	mannitol	MgSO/
1–5	11.2	12.0	16.4	12.3
5–3	5.3	6.6	4.0	7.1
2–5	4.6	2.6	7.8	4.8
4–3	2.3	1.4	1.75	3.7
2–4	18.6	23.0	21.0	10.3

From the fact that the salt uptake with increasing water uptake, also in the suction tension range where changes in the water conductivity can no more be expected, continues rising, we must conclude that the conductivity is not the only factor determining the salt uptake, but that beside it a passive component of the ion uptake directly linked to the waterstream occurs. According to table V this

would correspond with an influx coefficient of 1.4–3.7 %. This percentage approaches the influx coefficient, as it was found after the use of inhibitors (table IV). From these experiments it would appear that about 70–85 % of the ions in the transpiration stream is landed into it via a mechanism dependent on metabolism and that the correlation between water uptake and salt uptake, which is found here too is based on an increased salt uptake as a result of an increased conductivity for ions. In literature different cases are known from which the turgescence is deemed to affect the water conductivity (BREWIG 1937, 1939, ROSENE 1941, PERIS 1936, BROUWER 1953, 1954) but that this would also be the case for ion transport appears besides this investigation and the one published in 1954, from the bleeding experiments of VAN NIE, HELDER and ARISZ (1950), ARISZ, HELDER and VAN NIE (1951) and from researches by ARISZ and SCHREUDER (1956).

Moreover it appears from the experiments described here that part of the ions is landed into the transpiration stream by a mechanism not controlled by metabolism, i.e. it is not to be inhibited by a protracted treatment with inhibitors. May be this is a component of the ion uptake directly linked to the waterstream. Quantitatively this component is small in *Vicia faba* with respect to the active component.

If we survey the whole ion uptake in these experiments we can distinguish three components all of which are influenced by the water uptake.

A. The accumulation in the root increases with rising water uptake (fig. 20). Inhibitors reduce the accumulation (figs 16 and 21), whereas osmotica sucrose, lactose, mannitol as well as magnesium sulphate show an increase. Both the increase due to an increased water uptake and the one caused by osmotica indicates an easier penetration of the ions into the root.

B. As has been explained above the bleeding process is in these experiments the most important factor in the supply of ions for the transpiration stream. The strengthening of this process by the transpiration stream may be partly based on the concentration in the xylem vessels being constantly kept low (Hoagland) but on the other hand it shows so great a correlation with changes in conductivity, that an explanation at the hand of this phenomenon is obvious.

C. A component linked direct to the water stream which in Hylmö's experiments must be taken as the most important factor for the determination of the concentration in the transpiration stream, is present here, but acts a very minor part. This "passive" influx coefficient amounts here to 2–5 % of the medium concentration. This is the concentration which cannot be further reduced by the ruling out of metabolic processes. It seems therefore that only $\pm$ 20 % of the total ion transport to the shoot is brought about in this way.

In the whole ion transport we have therefore this basis concentration,

which is more or less increased by the bleeding mechanism (cf. HUBER 1953). No increase appears after a protracted treatment with inhibitors, a strong increase when sugar is administered. We might wonder whether an uptake mechanism on account of its dependence on metabolism should be called active or not. From a viewpoint of energy it is clear that energy is required for all those processes in which the concentration in any part of the plant grows higher than the one in the medium solution. With the Rb accumulation in the root this is the case. The question remains whether the ion transport to the xylem vessels is passive or active. It is a fact this transport can be retarded by inhibitors and accelerated by sucrose and other osmotica. This, however, also obtains for the water transport, be it at higher concentrations of the inhibitors. Yet we assume that the water uptake, at least in *Vicia faba*, is an almost completely passive process. With the influence of inhibitors on the water uptake we think of a change in conductivity. This possibility must also be left open for the ion uptake. Penetration then may be a question of diffusion, possibly accelerated diffusion. This penetrating is easier on application of osmotica, more difficult when inhibitors are used. Words as changes in permeability or changes in conductivity may then be used. They do not do more, however, than giving a name to a phenomenon that is in any case controlled by the living protoplasm. For the present, however, we can share the general conception and call this salt uptake dependent on metabolism active.

SUMMARY

The relation between water uptake and ion uptake has been investigated at various experimental conditions using 5–7 weeks old *Vicia faba* plants. Taking the uptake from a calcium chloride solution as a control, the relation between ion uptake and water uptake could be modified by using sugars and inhibitors. Applying sugars to the nutrient solution the concentration of the transpiration stream could be enhanced. Using inhibitors this concentration decreased.

The concentration of the transpiration stream amounted to about 13 % of the concentration in the outer solution on aerated calcium chloride solution, to about 24 % on calcium chloride solutions with sugar addition and to about 2–4 % using dinitrophenol addition or a non-aerated solution.

It is assumed that these facts indicate that a dominant part of the ions in the transpiration stream arrives there by means of a process dependent on metabolism. Only a small percentage of the total ion transport seems to be due to a passive carrying along with the transpiration stream.

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